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CONTENTS

January 2024, Volume 11, No.1

Title	Page
Investigation of charged particles interaction with CeBr3 scintillator by Monte Carlo simulation programs *Chalermpon Mutuwong, Wuttichai Chaiphaksa, Jakrapong Kaewkhao, Cherdsak Bootjomchai*	01 – 06
Evaluating the Effectiveness of English Speaking Conversation Practice for Communication Suitable for Students using the Random Forest Technique: A Case Study of Students Enrolled in English for Communication Course at Rajamangala University of Technology *Paveena Meteevorakij, Piyatida T.Chaisuwan, Kulwadee Tanwong*	07 – 19
Effect of Bioactive Peptide, KR-12-a5, on Growth of Clinical Methicillin-resistant *Staphylococcus aureus* Isolates *Chayaporn Saranpuetti, Phanthipha Phuttamek, Chonchanok Muangnapoh, Hathaitip Sritanaudomchai*	20 – 28
Microbiological Assessment of Car Doors and Steering Wheels at Benue State University, Makurdi: Public Health Implications *Olajide Akinnibosun, Peter Adikwu, Onyemowo Sunday David, Godwin Emmanuel Oyiwona, Paul Igomu Adole, Doowuese Yandev, Dominic Agbo Oche, Augustine Ameh Agbo, Sonia Onyemowo Ochekpe, Martina Enyanwu Okoh, Solomon Terser Soom, Amina Ojochide Hassan*	29 – 36

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Suan Sunandha Science and Technology Journal

Investigation of Charged Particles Interaction with $CeBr_3$ Scintillator by Monte Carlo Simulation Programs

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Abstract
This work aims to investigate the interaction of charged particles (alpha and proton) with the $CeBr_3$ scintillator by using Monte Carlo simulation. The total mass stopping power (TMSP), projected range, ion distributions, and ion ranges in the $CeBr_3$ at an energy range of 0.01 MeV - 10,000 MeV, were computed by SRIM and FLUKA programs. The simulation results show that the TMSP of CeBr3 obtained by both programs is in good agreement. The alpha particle has a higher TMSP of the $CeBr_3$ than the proton. The projected range of alpha and proton particles increases with increasing energy. The projected range of the proton is higher than that of the alpha particle when compared at the same energy. Finally, the 2D visualization of ion distributions and ion ranges for alpha and proton particles was reported.

Keywords: $CeBr_3$ scintillator, Monte Carlo simulation, Alpha, Proton

1. Introduction

Scintillators are materials that can emit visible light when interacting with radiation. Nowadays, scintillators have been continually used in industrial, medical, and scientific applications. Inorganic scintillators such as $CeBr_3$ are interesting due to high density and high-energy resolution of gamma-ray spectroscopy at room temperature (Idoeta, Herranz, Alegría, & Legarda, 2021). In recent years, many researchers have examined the fundamental characteristics of $CeBr_3$ detectors such as energy resolution (Naqvi, Khiari, Liadi, Khateeb-ur-Rehman, & Isab, 2016), pulse linearity (Giaz et al., 2015) and timing properties (Swiderski, Moszynski, Syntfeld-Kazuch, Szawlowski, & Szczesniak, 2014). There is no information on charged particles interacting with $CeBr_3$ scintillators. Therefore, this work aims to study charged particles interacting with $CeBr_3$ scintillators by using Monte Carlo simulation programs.

2. Theory

2.1 Mass stopping power

When the charged particles pass through matter, they lose energy by transferring their energy to excite and ionize the atoms or molecules of matter. The properties of materials that cause charged particles to lose energy per depth per density is called "Mass stopping power", as described by the Bethe-Bloch formula (Braibant, Giacomelli, & Spurio, 2012):

$$-\frac{1}{\rho}\frac{dE}{dx} = 2\pi N_A m_e r_e^2 c^2 \frac{Z}{A}\frac{z_e^2}{\beta^2}\left[\ln\left(\frac{2m_e\gamma^2 v^2 W_{max}}{I^2}\right) - 2\beta^2 - \delta - 2\frac{C}{Z}\right] \quad (1)$$

where

$r_e = e^2/m_e c^2$ classical electron radius
$2\pi m_e r_e^2 c^2$ = 0.1535 MeV $g^{-1}cm^2$

m_e= electron mass = $9.110 \cdot 10^{-31}$ kg
N_A = Avogadro's number = $6.022 \cdot 10^{23}$ mol^{-1}
I = mean ionization(*excitation*) potential of target
ρ = material density
Z = atomic number of absorber medium
A = atomic weight of absorber medium
z_e= charge of the incident particle
$\beta = v/c$ of incident particle
$\gamma = 1/\sqrt{1-\beta^2}$
δ = density effect correction (vital at high energy)
C = shell correction (already vital at low energy)
W_{max} = maximum kinetic energy imparted to an e^- in a single collision $\cong 2m_ec^2(\beta\gamma)^2$, for $M \gg m_e$.

2.2 Monte Carlo simulation software

Monte Carlo method is one of the old and most effective techniques for solving generally complex problems involving particle transport and interactions with matter in complex geometries (Ahdida et al., 2022). Nowadays, the software based on this methodology, such as FLUKA (Battistoni et al., 2015), GEANT4 (Allison et al., 2016), MCNPX (Vahabi & Shamsaie Zafarghandi, 2020), PHITS (Sato et al., 2018), and SRIM (Ziegler, Ziegler, & Biersack, 2010), has been widely used to simulate the particle transport and interactions with matter. In this work, the charged particles interaction with $CeBr_3$ scintillators were investigated by SRIM and FLUKA Monte Carlo simulation programs. The weight fraction, and density of $CeBr_3$ are Ce(0.3689) and Br(0.6311), and 5.2 g/cm^3, respectively. The SRIM and FLUKA were used to calculate the total mass stopping power (TMSP) of the $CeBr_3$ for alpha and proton particles in the energy range of 0.01 MeV – 10,000 MeV. Moreover, the projected range, ion distribution, and ion ranges of the $CeBr_3$ for alpha and proton particles were investigated by SRIM. The simulation was run with a total of 10,000 primary particles.

3. Results and Discussion

The TMSP, projected range, ion distribution, and ion ranges of the $CeBr_3$ scintillator for alpha and proton particles were investigated using SRIM and FLUKA programs. The TMSP of $CeBr_3$ in energy range of 0.01 MeV- to 10,000 MeV for a) alpha and b) proton was illustrated in Figure 1.

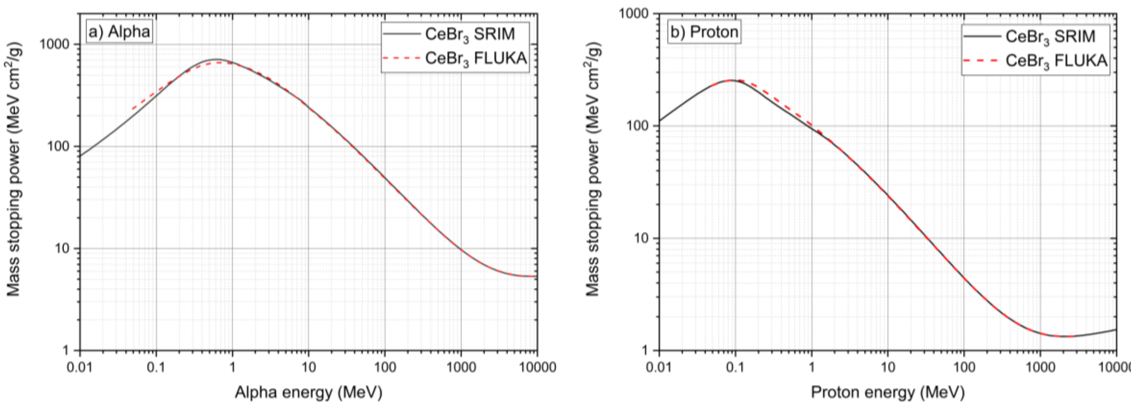


Figure 1. Mass stopping power of $CeBr_3$ for a) alpha and b) proton particles in energy ranges from 0.01 MeV to 10,000 MeV computed by SRIM (solid line) and FLUKA (dashed line).

The results found that the TMSP of the $CeBr_3$ scintillator computed by SRIM and FLUKA is in good agreement. The maximum values of TMSP are approximately 711.50 MeV cm^2/g at an alpha energy of 0.65 MeV and 256.35 MeV cm^2/g at a proton energy of 0.10 MeV, respectively. Due to the energy dependence of the energy loss (or total mass stopping power curve), incoming high-energy particles experience a negligible energy loss dE/dx, whereas the energy loss reaches its maximum when the particles have slowed to energies that correspond to the peak of the energy loss curve (El-Ghossain, 2017). In addition, the TMSP of the alpha particle tends to be higher than that of the proton as increasing energy. This effect is a result of the fact that the alpha

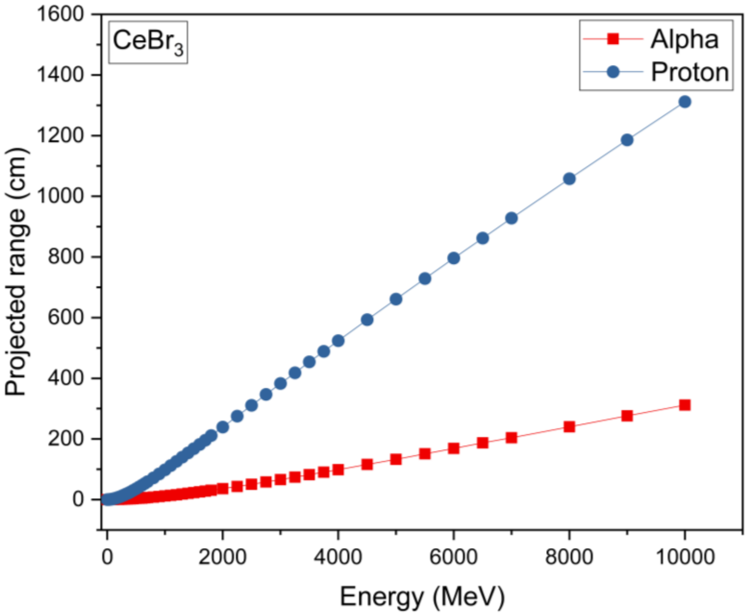
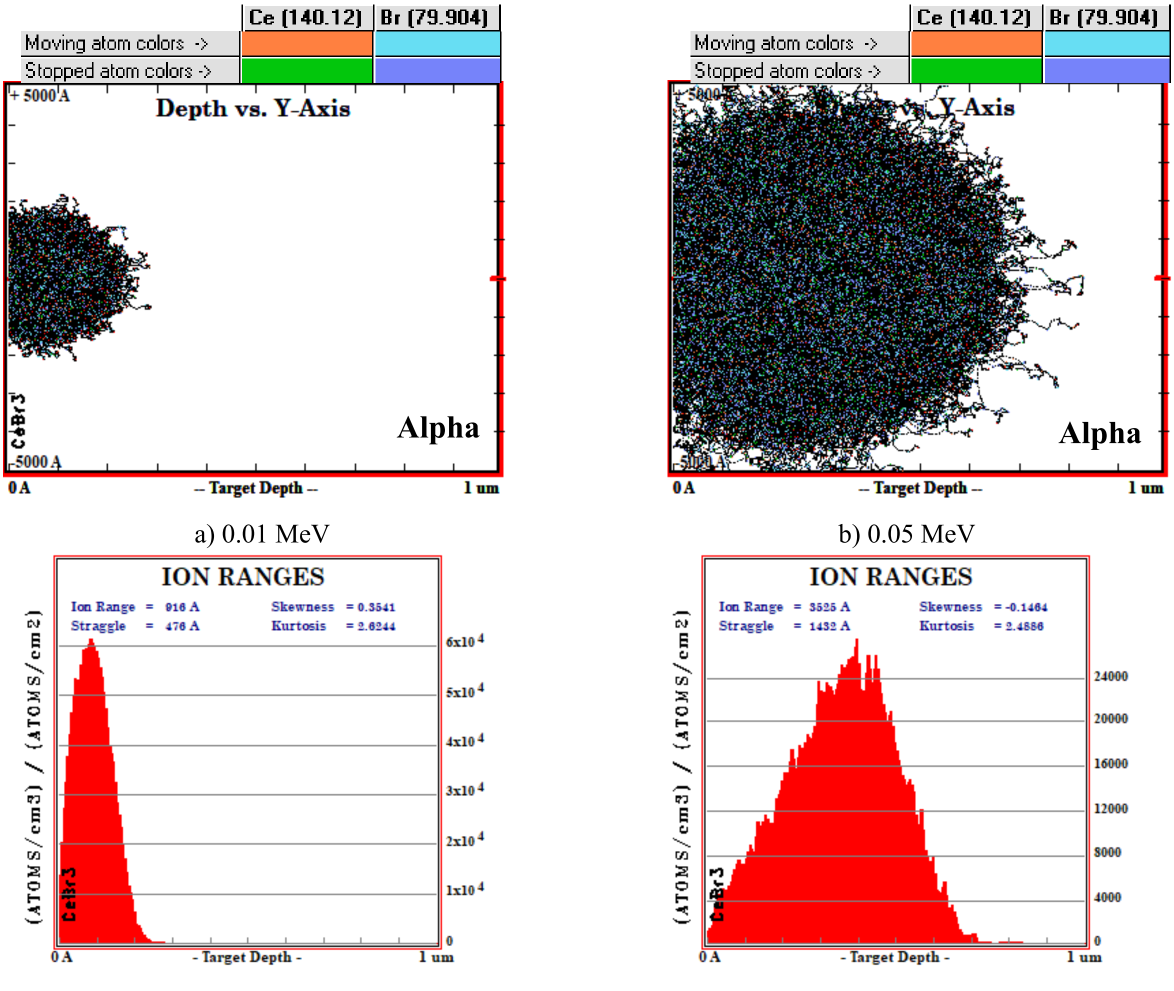


Figure 2. The projected range of alpha and proton particles in $CeBr_3$ from 0.01 MeV to 10,000 MeV.

particle has a lower penetration power than the proton. This is because the TMSP value of alpha particle is more than that of proton particle. The TMSP indicates the potentiality of a material to reduce the kinetic energy of particles traveling toward it by ionizing the molecules. The average penetration depth that particles stop in the material is called the “projected range”.

Error! Reference source not found.The average penetration depth of alpha and proton into $CeBr_3$ as a function of their kinetic energy was shown in Figure 2. The results indicate that the projected range of alpha and proton particles penetrating the $CeBr_3$ increases with increasing energy. Moreover, because proton particles have greater penetration power than alpha particles, the projected range of the proton particles is greater than that of the alpha particles when compared with the same energy.

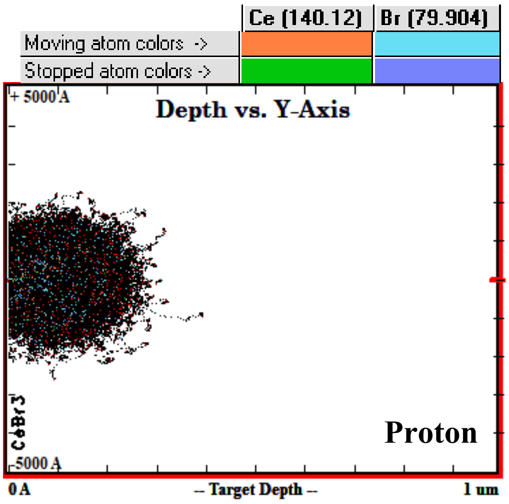
The 2D simulation of the ion distribution when alpha particles interact with $CeBr_3$ at energies of a) 0.01 MeV and b) 0.05 MeV is shown in Figure 3a and 3b, respectively. The alpha particles lose their energy by transferring energy to $CeBr_3$. The alpha particle has enough energy to ionize the Ce and Br atoms. These ions and alpha still move into $CeBr_3$ until their movement are stopped. The orange line traces the Ce ion trajectory until it stops at the green dot. The blue line traces the movement path of the Br ion until it stops at the purple dot. The black line is the trajectory of alpha particles to stop in $CeBr_3$ at the red dot. The simulation results showed the ion range distribution of alpha particles into $CeBr_3$ is between 0 angstrom and 0.28 µm for incident alpha energies of 0.01 MeV (Figure 3c) and between 0 angstrom and 0.84 µm for incident energies of 0.05 MeV (Figure 3d). The average ion ranges of alpha particles are about 916 angstroms or 0.0916 µm for 0.01 MeV, as shown in Figure 3c, and about 3525 angstroms or 0.3525 µm for 0.05 MeV, as illustrated in Figure 3d.



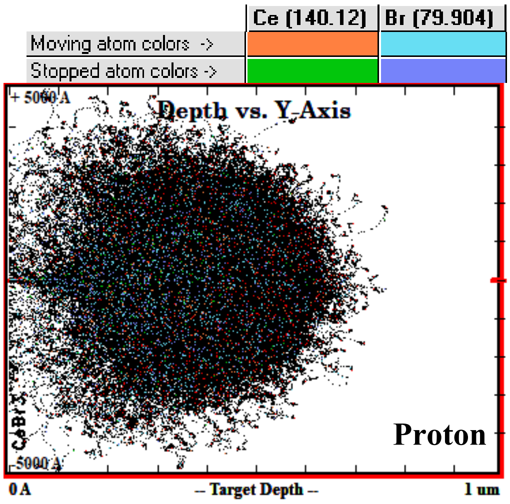
a) 0.01 MeV b) 0.05 MeV c) 0.01 MeV d) 0.05 MeV

Figure 3. The 2D Monte Carlo simulation of the 10,000 alpha particles interact with the $CeBr_3$ at energies a) 0.01 MeV, b) 0.05 MeV, and the probability distributions of ion ranges at c) 0.01 MeV and d) 0.05 MeV.

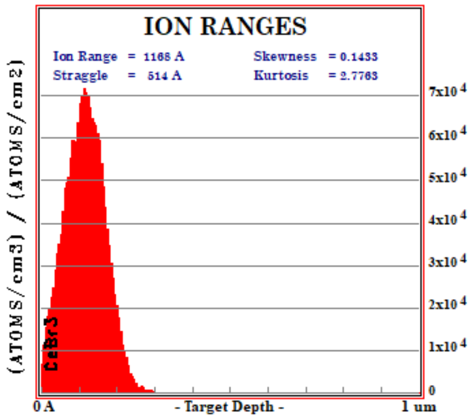
The results of the 2D simulation of the ion distribution produced by proton particle interactions with $CeBr_3$ at energies of 0.01 MeV and 0.05 MeV were shown in Figure 4a and 4b, respectively. According to the simulation results, the ion range distribution of proton particles into $CeBr_3$ is between 0 angstrom and 0.30 µm for incident energies of 0.01 MeV (Figure 4c) and between 0 angstrom and 0.74 µm for incident energies of 0.05 MeV (Figure 4d). The average penetration depth of proton particles is about 1168 angstroms or 0.1168 µm for 0.01 MeV, as shown in Figure 4c, and about 4224 angstroms or 0.4224 µm for 0.05 MeV, as shown in Figure 4d.



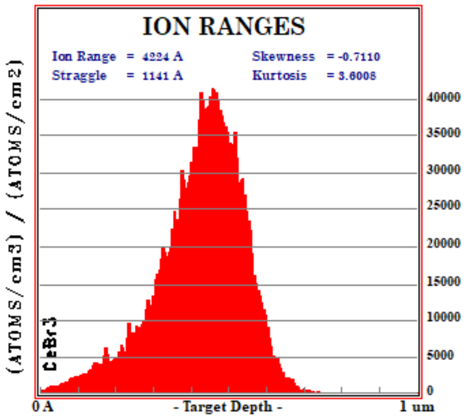
a) 0.01 MeV



b) 0.05 MeV



c) 0.01 MeV



d) 0.05 MeV

Figure 4. The 2D Monte Carlo simulation of the 10,000 proton particles interact with the $CeBr_3$ at energies a) 0.01 MeV, b) 0.05 MeV, and the probability distributions of ion ranges at c) 0.01 MeV and d) 0.05 MeV.

4. Conclusion

The TMSP, projected range, ion distribution, and ion ranges of alpha and protons interacting with the $CeBr_3$ scintillator in the energy range of 0.01 MeV - 10,000 MeV were studied by using SRIM and FLUKA software. The results found that the TMSP of the $CeBr_3$ scintillator for alpha and proton is in excellent agreement. The TMSP of the alpha particle tends to be higher than that of the proton. The projected range of alpha particles and protons in the $CeBr_3$ increases with increasing energy. The projected range of the proton is higher than that of the alpha particle when at the same energy. Finally, the 2D simulation of ion distributions and ion ranges for alpha and proton particles was reported. The average ion ranges of alpha particles are 0.0916 μm for 0.01 MeV and 0.3525 μm for 0.05 MeV. For proton particles, the average ion ranges are 0.1168 μm for 0.01 MeV and 0.4224 μm for 0.05 MeV.

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References

Ahdida, C., Bozzato, D., Calzolari, D., Cerutti, F., Charitonidis, N., Cimmino, A., ... Widorski, M. (2022). New capabilities of the FLUKA multi-purpose code. *Frontiers in Physics*, *9*, 788253. doi:10.3389/fphy.2021.788253

Allison, J., Amako, K., Apostolakis, J., Arce, P., Asai, M., Aso, T., … Yoshida, H. (2016). Recent developments in GEANT4. *Nuclear Instruments and Methods in Physics Research, Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*, *835*, 186-225. doi:10.1016/j.nima.2016.06.125

Battistoni, G., Boehlen, T., Cerutti, F., Chin, P. W., Esposito, L. S., Fassò, A., ... Smirnov, G. (2015). Overview of the FLUKA code. *Annals of Nuclear Energy*, *82*, 10-18. doi:10.1016/j.anucene.2014.11.007

Braibant, S., Giacomelli, G., & Spurio, M. (2012). *Particles and fundamental interactions*. Dordrecht: Springer.

El-Ghossain, M. O. (2017). Calculations of stopping power, and range of ions radiation (alpha particles) interaction with different materials and human body parts. *International Journal of Physics*, *5*(3), 92-98.

Giaz, A., Hull, G., Fossati, V., Cherepy, N., Camera, F., Blasi, N., ... Riboldi, S. (2015). Preliminary investigation of scintillator materials properties: SrI_2:Eu, $CeBr_3$ and GYGAG:Ce for gamma rays up to 9 MeV. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*, *804*, 212-220. doi:10.1016/j.nima.2015.09.065

Idoeta, R., Herranz, M., Alegría, N., & Legarda, F. (2021). Possibilities of the use of $CeBr_3$ scintillation detectors for the measurement of the content of radionuclides in samples for environmental monitoring. *Applied Radiation and Isotopes*, *176*. doi:10.1016/j.apradiso.2021.109881

Naqvi, A. A., Khiari, F. Z., Liadi, F. A., Khateeb-ur-Rehman, & Isab, A. A. (2016). Performance tests of a large volume cerium tribromide ($CeBr_3$) scintillation detector. *Applied Radiation and Isotopes*, *114*, 50-56. doi:10.1016/j.apradiso.2016.04.031

Sato, T., Iwamoto, Y., Hashimoto, S., Ogawa, T., Furuta, T., Abe, S. I., ... Niita, K. (2018). Features of particle and heavy ion transport code system (PHITS) version 3.02. *Journal of Nuclear Science and Technology*, *55*(6), 684-690. doi:10.1080/00223131.2017.1419890

Swiderski, L., Moszynski, M., Syntfeld-Kazuch, A., Szawlowski, M., & Szczesniak, T. (2014). Measuring the scintillation decay time for different energy depositions in NaI:Tl, LSO:Ce and $CeBr_3$ scintillators. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*, *749*, 68-73. doi:10.1016/j.nima.2014.02.045

Vahabi, S. M., & Shamsaie Zafarghandi, M. (2020). Applications of MCNP simulation in treatment planning: A comparative study. *Radiation and Environmental Biophysics*, *59*(2), 307-319. doi:10.1007/s00411-020-00841-2

Ziegler, J. F., Ziegler, M. D., & Biersack, J. P. (2010). SRIM–The stopping and range of ions in matter (2010). *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, *268*(11-12), 1818-1823. doi:10.1016/j.nimb.2010.02.091

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Evaluating the Effectiveness of English Speaking Conversation Practice for Communication: A Case Study of Students Enrolled in the English for Communication Course at Rajamangala University of Technology Tawan-ok Using the Random Forest Technique

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Abstract
The objectives of this research were twofold: 1) to compare the effectiveness before and after using a dialogue-based English speaking skills training model for effective communication, and 2) to identify guidelines for adjusting the English speaking skills training model using the random forest technique suited to students enrolled in the English for communication course at Rajamangala University of Technology Tawan-ok. The tools used in this research were 1) a dialogue-based English speaking skills assessment questionnaire, and 2) observation of English speaking behaviour for communication. The research findings indicated that the average scores before implementing the speaking skills training model were 3.11, which was considered good. After the training, the average scores increased to 4.30, indicating a significantly higher level of proficiency. The statistical test yielded a t-value of 15.871. Therefore, it could be concluded that the English speaking skills training model significantly enhanced students' English communication abilities at a significance level of .05. Regarding the guidelines for adjusting the English speaking skills training model using the random forest technique, the data classification results demonstrated an accuracy rate of 86.78%, a recall rate of 85.4%, and a precision rate of 83.13%. Additionally, 18 rules were identified as having significant correlations. The adjusted training model focused on various topics, including Greetings, Feelings and Emotions, Health, Asking for and Giving Directions, Weather, and University Life.

Keywords: Efficiency evaluation, Dialogue-based English speaking skills training, Data mining, Random forest technique

1. Introduction

From Article 34 of the ASEAN Charter, which states, 'The working language of ASEAN shall be English,' the English language plays a crucial role as an international language, emphasizing its use in government affairs and private sector businesses. This has been the case for almost ten years since 2012 (Ruksuan, 2022). Despite the awareness that English is a tool for academic success and increasing opportunities in the workforce, the proficiency level of English language communication among Thais remains significantly low, as evident from the results of English language proficiency exams. Thailand's English language skills are ranked lower than other countries in the Asian region. According to the EF English Proficiency Index (2022), which measures language proficiency through online tests with a sample group of at least 400 individuals, English language skills in Thailand are at a very low proficiency level. In terms of English proficiency, Thailand ranks 21st out of 24 countries in the Asian region, according to the EF English Proficiency Index (2022). The factors that contribute to low English language proficiency include individual characteristics, teaching techniques, and national educational policies (Hengsuko, Boonkao, Srihanam, & Manee, 2019). Additionally, there is a research study by Tangpattanakit (2020) that examines the influence of factors on English language communication. It found that basic knowledge, learning strategies, and individual attitudes contribute to English language communication. Therefore, it is necessary to explore teaching methods that enable learners to communicate in English in their daily lives. Teachers have the responsibility to encourage learners to express themselves in the language, utilize language activities as a tool for practice, and incorporate real-life situations to aid in the learning process. The statement is consistent with Rujichom (2018), which stated that communication skills, particularly speaking skills, are essential as they are used more frequently in daily life than other skills. Speaking ability facilitates the acquisition of other skills and contributes to better interpersonal interactions. Additionally, Ur (1996) emphasizes the importance of speaking skills as they demonstrate and reveal the speaker's knowledge and understanding of the language. Indeed, it is necessary to have standardized criteria to assess individuals' speaking skills in order for them to understand their own development. This is why individuals need to be aware of their initial level of English language communication skills to facilitate their appropriate personal development. It enables them to select suitable materials and processes for improvement.

Testing English-speaking skills can indeed improve students' communication abilities. When students are assessed on their speaking skills, it prompts them to actively use and apply their language knowledge. This process helps identify strengths and weaknesses, encourages practice and preparation, and ultimately leads to an enhancement in speaking proficiency through consistent assessment and practice. Therefore, testing English speaking skills for communication should include pre-training and post-training assessments to compare the effectiveness of the media and the process used. This objective measurement should be complemented by statistical methods to analyze whether learners' abilities have significantly improved compared to the traditional approach.One statistical test that can be used is the Independent-Sample t-test, which tests the hypothesis between two independent sample groups. This test determines if there is a significant difference in the means of the sample groups. The preliminary requirement is that the two sample groups must be independent and obtained through random sampling from a population with normally distributed variables. Additionally, the variable being tested should be on an interval or ratio scale. Alternatively, a Paired-Sample t-test can be used to compare data before and after within the same sample group. The preliminary requirement for this test is that there should be one sample group, obtained through random sampling from a population with normally distributed variables. The variable being tested should be on an interval or ratio scale, and the independent variable should be related to the dependent variable (Kanjanawasee, Pitayanon, & Srisukho, 2008). However, the statistical measurements mentioned only provide information about the difference in English communication skills before and after the intervention. If we want to delve deeper into the content that was used to enhance skills and design learning strategies for other groups, researchers may consider utilizing data mining techniques to further enhance the clarity of the research findings and improve the reliability of their applications. For example, the research by Meteevorakij, Chaisuwan, Nubpetchploy, and Tanwong (2020) using decision trees to predict English language communication abilities for business communication based on 21st-century learning skills had an accuracy of 76%. Additionally, there is a study by Huynh-Cam, Chen, and Le (2021) that used decision trees and random forest algorithms to predict comprehensive teaching status. They predicted indicators to forecast performance for strategic planning in teaching management before the start of a new academic term. The study found that the prediction accuracy was 79.99% for random forest and 74.59% for decision trees. This research aligns with the study conducted by Madaan, Kumar, Keshri, Jain, and Nagrath (2021), which compared random forest and decision trees on the same dataset. The conclusion drawn from the results was that the random forest algorithm outperformed decision trees in terms of accuracy. It is a method of organizing information into nodes and branches. Each node represents a feature test. And the results of the test depend on the specified characteristics or independent variables. Building this model involves dividing data into groups based on specified conditions. Continue this process until you have data groups that correspond to the target variables you want to predict. If the amount of data is too small, this can cause the model to be unable to accurately predict data that has not been encountered before and cause overfitting. Although decision trees can be straightforward to interpret, random forest focuses on diverse features and variations that occur, enabling it to capture complex feature patterns more effectively than decision trees. Additionally, it reduces the risk of overfitting by preventing excessive reliance on training data. This contributes to enhancing prediction accuracy. This perspective is supported by the research of Denisko and Hoffman (2018). Therefore, researchers have adopted these techniques to aid in the analysis of measuring the effectiveness of English conversation training for communication using the random forest technique. Random forest is a data mining technique that combines multiple non-repetitive decision trees to address the inflexibility of individual decision trees and reduce overfitting issues. (Jayaprakash, Krishnan, & Jaiganesh, 2020). Moreover, it is a machine learning technique that can be used for classification, regression, and other tasks. It creates multiple decision trees during training and outputs the class selected by most trees for classification tasks or the mean or average prediction of the individual trees for regression tasks. Random Forest is a popular algorithm due to its simplicity, flexibility, and ability to handle both classification and regression problems. This is consistent with the research conducted by Kaewpanitch (2020), which found that the model developed using the random forest technique provided higher accuracy in predicting the performance of students in English language learning compared to the decision tree technique.

This can help in adjusting the topics of English conversation training for communication to be more suitable for students studying English for Communication at Rajamangala University of Technology Tawan-ok. It also enables instructors to plan their teaching and learning strategies in English language courses for communication that are tailored to future learners.

2. Research Objective

2.1 To compare the effectiveness of a dialogue-based English speaking skills training model for communication purposes before and after its implementation.

2.2 To identify guidelines for adapting the dialogue-based English speaking skills training model, using the random forest technique, to cater to the specific needs of students enrolled in the English for communication course at Rajamangala University of Technology Tawan-ok.

3. Research Scope

3.1 The research scope of this study included the population of 150 students enrolled in the English for Communication course during the first semester of the academic year 2022 at Rajamangala University of Technology Tawan-ok Bangphra Campus. Subsequently, the sample size was calculated using the G*power program version 3.1.9.7. The size of the effect (Effect size) was set 0.5, the significance level α (α err prob) is 0.05 and level of power (Power (1-β err prob)) is 0.95. Due to the lack of knowledge regarding estimated parameters from previous studies, the approach of specifying a standardized effect size was chosen. The medium effect size 0.5 (Cohen, 1988) was selected, resulting in a minimum total sample size of 45 individuals. Therefore, this research determined the sample size to be 50. The study sample consisted of 50 individuals who registered for the English for Communication course during the same semester. The sample group was selected through purposive sampling. This method was used because the researchers need the sample group that had similar basic knowledge of English language and to avoid bias that could arise from different evaluators. Then simple random sampling was used subsequently. The research was conducted at the Bangphra campus (The Office of Academic Support and Registration Rajamangala University of Technology Tawan-ok, 2005).

3.2 The research duration involved conducting the study with students enrolled in the English for Communication course at Rajamangala University of Technology Tawan-ok, Bangphra Campus, during the first semester of the academic year 2022. The study commenced in May 2022 and concluded in September 2022, with a total of 50 participants.

3.3 The research tools used in this study included.

3.3.1 The dialogue-based English speaking skills training model for communication, which comprised six topics: Greeting-Basic, Feeling and Emotion, Weather, Health, University Life, and Asking and Giving Directions. These topics were derived from a referenced textbook by Beatty, Longshaw, and Austin (2020). For each topic, students received materials from the instructor to practice and apply independently.

3.3.2 The observation of English speaking behavior for communication, which encompassed three aspects: Pronunciation, Grammar, and Fluency. These aspects were based on the core lessons and criteria developed following Clark's framework (Stansfield, 1973). The criteria were divided into four levels, ranging from beginner to advanced proficiency. The levels are as follows:

(1) Pronunciation: speaking or responding with accurate pronunciation.

(2) Grammar: speaking without grammar mistakes and using vocabulary and grammar structures accurately.

(3) Fluency: speaking conversationally with natural fluency.

3.3.3 Comparing the proficiency scores in English speaking skills for communication using the Likert scale criteria of 4 levels.

4. Research Framework

Based on the theories and concepts of Carroll (1980), Ellis and Johnson (1994), individuals with a good knowledge of the English language may still struggle to respond or communicate effectively when asked for directions or in various social situations. This can be considered a failure of the Human Introduction Processing System in language learning, particularly in English. Therefore, learners need both knowledge and practice in using English to develop proficiency, especially in speaking skills. Many students begin learning English in secondary school but are unable to communicate effectively with native speakers, which leads to a lack of confidence and hinders their progress in professional or higher-level learning contexts. Dell Hymes, a sociolinguist from the United States, highlights the significance of communicative competence, which focuses on enabling language learners to use the language appropriately in diverse social contexts. This approach equips learners with language knowledge and skills simultaneously, enabling effective progress in their careers or further studies (Richards & Rodgers, 2002).

For this research, the researchers have integrated both of the aforementioned frameworks and applied them to examine the development of English language communication abilities among students at Rajamangala University of Technology Tawan-ok.

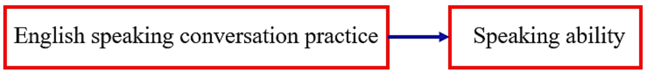

Figure 1. Conceptual Framework.

5. Research Methodology

This research employed a quasi-experimental design, specifically a one-group pretest-posttest design. The researchers outlined the following steps for conducting the study:

5.1 Participants: The study involved 50 students who were enrolled in the English for Communication course during the first semester of the 2022 academic year.

5.2 Process of creating and finding the quality of tools involved the following steps:

5.2.1 Conversation Speaking Exercise:

(1) Reviewed relevant documents and studies on speaking activities for communication purposes, such as "A study of out-of-class English language learning activities of secondary school students in English Program" by Wiengnil (2010) and the book "Practice Makes Perfect: English Conversation Premium, Second Edition" by Yates (2016).

(2) Created an English conversation speaking exercise based on the curriculum and content framework of the English Communication course. The exercise focused on 6 topics: Greeting-Basic, Feeling and Emotion, Weather, Health, University Life, and Asking and Giving Directions.

5.2.2 Created an observation form for English speaking behavior for communication purposes. The form utilized a pretest and posttest format and established assessment criteria based on Clark's evaluation framework.

5.2.3 Assessed the content validity and the measurement and evaluation of the training and observational behavior of English communication skills by experts. Calculated the Index of Congruence (IOC) based on the analysis conducted by three experts. The results showed that the average IOC values for all items were greater than 0.6, indicating that the training materials were appropriate and aligned with the objectives (Thaweerat, 1997).

5.3 Data collection was conducted by the researchers following the sequential steps below:

5.3.1 A pretest was conducted using an oral test format, consisting of 6 topics, to assess English speaking skills for communication. The topics were provided along with vocabulary prompts and applicable sentence structures. The evaluation was performed by two assessors, including one foreign language instructor and one researcher, totaling two assessors.

5.3.2 The researchers conducted the experiment using a training model consisting of 6 dialogue-based topics for English speaking skills in communication. The topics were arranged in the following order: Greeting-Basic, Feeling and Emotion, Weather, Health, University Life, and Asking and Giving Directions. The researchers taught the vocabulary and grammatical structures to the students. The students then applied the vocabulary they had learned into the grammatical structures they had studied. Next, the researchers asked the students to read and translate the created conversation sentences, and the researchers explained the pronunciation, translation and overall meaning of the sentences. Finally, the students were provided suggestions for creating new sentences using the vocabulary and grammatical structures learned. The training emphasized practice and transfer of knowledge, with one topic taught per week, following the order of the aforementioned topics. The process workflow was as follows:

(1) Introduced Topic 1: Greeting-Basic.

(2) Explained the content of the lesson before providing the practice exercises.

(3) The sample group practiced the dialogue exercises for a duration of 1 week.

(4) At the end of the week, the performance of the dialogue practice in that specific topic was assessed.

(5) Continued this process, sequentially moving through all 6 topics without repeating any previous topics.

The sample group had freedom to independently engage in the training process with the assigned lesson content.

5.3.3 The posttest assessment involved using the same conversational English practice materials as the training. Each individual was assessed through an oral test. This test was conducted after the learners had practiced each lesson on their own.

5.3.4 The scores obtained from the pretest and posttest assessments, following Clark's framework, were averaged and compared to evaluate the proficiency in English speaking for communication purposes. The results were then translated using Likert's criteria for assessing levels of speaking proficiency in English.

5.4 In data analysis, the researchers conducted the following steps:

5.4.1 Descriptive statistics: Employed descriptive statistics to analyze the general data.

5.4.2 Compared pretest and posttest scores in English speaking ability using the Paired-Samples t-test and performed a preliminary assumption check:

(1) Examined whether the dependent variable, which is the posttest scores of English speaking ability following the use of the conversational training program, followed a normal distribution.

(2) Examined whether the independent variable, which is the pretest scores of English speaking ability prior to the use of the conversational training program, had a correlation with the dependent variable.

5.4.3 Adjusted the conversational training program for English speaking communication using the random forest technique, which involves combining multiple non-repetitive decision trees to work together.

(1) The process involved understanding the data, defining the attributes, and analyzing the data for the conversational training program for English language with 6 topics and the observation of English-speaking behavior across 3 dimensions.

(2) The Data Preparation Phase involves transforming the data into a format that is compatible with "Nodes" and lists the sample for each layer. During the creation of a decision tree, the objective is to select the attribute/feature with the lowest Gini index as the root node. The Gini coefficient has a range from zero to one. A value of zero represents a system of perfect equality across the population (all nodes are in the same class), while a value of 0.5 indicates distributed sampling. The equal data in the two "value" classes at the end come from samples that satisfy the output condition. The formula for the Gini index is as follows (Dorfman, 1979).

$$G_i = 1 - \sum_{k=1}^{n} p^2 i, k$$

'pi' represents the probability of an object being classified into a specific class.

pi,k denotes the proportion that indicates the ratio of the number of data instances in Node i belonging to Class k.

(3) Design: The random forest model utilizes ensemble learning, which combines multiple approximate value estimation models to reduce bias, variance, and data sensitivity.

(4) Dataset: The "S_Daily English Conver" dataset is processed using bootstrapping. Bootstrapping involves randomly sampling new subsets from the original dataset, replacing and increasing its size. This process helps generate sufficient data for model building. The dataset is then divided into training data and testing data following the principles of "k-fold Cross Validation (k-fold CV: k=10)." To conduct the model, researchers utilize data for training with a split of 90% for each round as training data, and a split of 10% is reserved as testing data for evaluation. This cross-validation technique assists in assessing the model's performance and its ability to generalize.

(5) Pseudo code for optimizing parameters using Grid Search (English Conversation Practice and English Speaking Behavior Observation: ECP_ESB)

Input: Database Sex, Chapter, Observation, Level Skill,

Output: Ranking Chapter

Method: # load sample data

```
Training-Data= “x_Eng C”
(x_Eng C_train, y_Eng C_train, z_Eng C_train,model)
While Attribute Num>0 Do
For Each Attribute Chap, Do
Sorted Data=Sort Training Data By (sex,)
sexAdvantagen [i] = Define Advantage (sex, SortedData)
 procedure division.
  display " " with blank screen.
  perform loop1 varying i from 1 by 1 until i > 5.
  perform loop2 varying i from 1 by 1 until i > 5.
  display "Max : " max.
      stop run.
      loop1.
  display i  " : " with no advancing.
       accept ar(i).
       loop2.
  if ar(i) > max
       move ar(i) to max.
End For …..
End While
```

(6) Preview: decision tree Models are used to predict the next response based on independent learning from each tree, such as the "Greeting-Basic" model shown in the example figure 2.

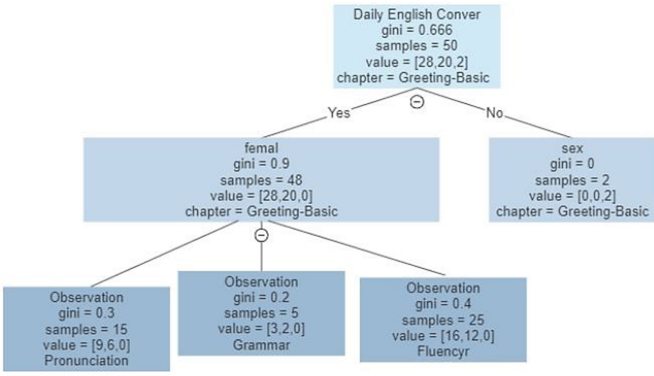

Figure 2. Illustration of the decision tree model "Greeting-Basic" creation.

(7) Evaluate the performance of the model: The accuracy of evaluated classification models is informally defined as the fraction of correct predictions made by our model. However, validity can be defined in the following ways:

$$Accuracy = \frac{Number\ of\ correct\ predictions}{Total\ number\ of\ predictions}$$

For binary classification, accuracy can also be calculated in terms of positive and negative values as follows:

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN}$$

where TP = True Positives, TN = True Negatives, FP = False Positives and FN = False Negatives. (Table 6)

6. The Results of the Data Analysis

The results of the data analysis, classified according to Objective 1, which aims to compare the effectiveness before and after using the English conversation training, are as follows:

Table 1. English communication abilities before and after using English conversation training.

Average Level	Meaning	Number		Percent	
		Before	After	Before	After
1.00-2.00	Low ability	8	-	16.00	-
2.01-3.00	Medium ability	16	-	32.00	-
3.01-4.00	Good Ability	12	20	24.00	40.00
4.01-5.00	Very good ability	14	30	28.00	60.00
Total		50	50	100.00	100.00

According to Table 1, the finding of English communication abilities before using English conversation training revealed that the majority of students in the sample group had a moderate level of English communication skills prior to the test (32.00%). The next highest category was a very good level (28.00%), followed by a good level (24.00%), and a low level (8.00%) of English communication skills prior to the test.

Moreover, it is evident that more than half of the sample group, specifically 60.00%, achieved a very good level of English communication abilities after the test. Additionally, a substantial portion, 40.00%, attained a good level of English communication abilities after using English conversation training.

Table 2. The average scores before and after using the English conversation training program for each section.

Test Results	Greeting-Basic	Feeling and Emotion	Weather	Health	University life	Asking and Giving Directions
Pretest	4.06	3.58	2.37	3.23	2.20	3.23
Posttest	4.86	4.70	3.96	4.41	3.66	4.20

According to Table 2, it is evident that the sample group of students had the highest average score in the "Greeting-Basic" section (4.06) before using the English conversation training program. The second highest average score was observed in the "Feeling and Emotion" section (3.58), followed by the "Health" and "Asking and Giving Directions" sections (3.23) respectively. The lowest average score was obtained in the "University Life" section (2.20).

Regarding the average scores after using the training program, the highest average score was achieved in the "Greeting-Basic" section (4.86), followed by the "Feeling and Emotion" section (4.70) and the "Health" section (4.41). The lowest average score was observed in the "University Life" section (3.66).

Table 3. The results of the post-training scores in English conversation speaking ability follows a normal distribution.

Test	Skewness		Kurtosis		Kolmogorov-Smirnov	
Results	**Statistic**	**Std. Error**	**Statistic**	**Std. Error**	**Kolmogorov-Smirnov Z**	**Sig**
Posttest	-.467	.337	-1.211	.662	1.343	.054

*p<.05

According to the results from Table 3, the post-training scores in English conversation speaking ability showed a distribution that was close to normal. This conclusion is supported by the values of Zskewness = -1.386, Zkurtosis = -1.829, and Kolmogorov-Smirnov Z = 1.343 (sig = 0.054). Therefore, we can conclude that the variable "post-training scores in English conversation speaking ability" exhibited a distribution that closely resembles a normal distribution (Hair, Black, Babin, & Anderson, 2019).

Table 4. The results of the evaluation show that the scores before and after using the conversational English speaking training program for communication.

Test results	N	Correlation	Sig.
Pair 1 Posttest & Pretest	50	.812	.000

*p<.05

Based on Table 4, it is evident that there exists a significant correlation between the scores before and after implementing the conversational English speaking training program for communication (r = .812, sig = .000). Consequently, considering the information presented in Tables 3 and 4, it can be concluded that the data aligns with the initial assumptions of the statistical analysis utilizing the Paired-Samples t-test (Kanjanawasee et al., 2008).

Table 5. Comparing the average scores before and after using the conversational English speaking training program for communication in the sample group.

Test Results	N	$\bar{x}$	SD	df	t	p
Pretest	50	3.11	0.87	49	15.871	.000
Posttest	50	4.30	0.57			

*p<.05

According to Table 5, it is evident that among the students in the sample group who enrolled in the "English for Communication" course during the 1st semester of the academic year 2022, there is a statistically significant difference in the average scores before and after utilizing the conversational English speaking training program. The calculated test statistic was 15.871, with degrees of freedom equal to 49, and a significance value of .000. Consequently, it can be concluded that following the training in conversational English speaking, there was a substantial enhancement in English language communication skills at a significance level of .05.

Regarding the analysis of data for Objective 2, which aims to establish guidelines for adapting the conversational English speaking training program for effective communication using the random forest technique, the following information is provided:

Table 6. The performance measurement of the conversational English speaking training data classification using the random forest technique.

	Pronunciation	Grammar	Fluency	**Precision**
Pronunciation	842	0	0	82.6%
Grammar	0	809	5	80.1%
Fluency	0	0	904	86.7%
Recall	85.2%	88%	83%	
Accuracy	86.78%			

According to Table 6, the performance measurements for the classification of conversational English speaking training data using the random forest technique are as follows: The accuracy rate was 86.78%, the recall rate was 85.4%, and the precision rate was 83.13%.

Table 7. The classification of conversational English speaking training data is done for each of the 6 topics that have an impact on improving the test scores of students majoring in English for international communication.

Data classification rules	Lesson
If Chap = 1 and Eva_Ob = 1 and Level = 4	Greeting-Basic
If Chap = 1 and Eva_Ob = 2 and Level = 3	Greeting-Basic
If Chap = 1 and Eva_Ob = 3 and Level = 4	Greeting-Basic
If Chap = 2 and Eva_Ob = 1 and Level =3	Feeling and Emotion
If Chap = 2 and Eva_Ob = 2 and Level = 3	Feeling and Emotion
If Chap = 2 and Eva_Ob = 3 and Level = 4	Feeling and Emotion
If Chap = 3 and Eva_Ob = 1 and Level = 2	Weather
If Chap = 3 and Eva_Ob = 2 and Level = 2	Weather
If Chap = 3 and Eva_Ob = 3 and Level = 3	Weather
If Chap = 4 and Eva_Ob = 1 and Level = 3	Health
If Chap = 4 and Eva_Ob = 2 and Level = 2	Health
If Chap = 4 and Eva_Ob = 3 and Level = 4	Health
If Chap = 5 and Eva_Ob = 1 and Level = 2	University life
If Chap = 5 and Eva_Ob = 2 and Level = 2	University life
If Chap = 5 and Eva_Ob = 3 and Level = 2	University life
If Chap = 6 and Eva_Ob = 1 and Level = 3	Asking and Giving Directions
If Chap = 6 and Eva_Ob = 2 and Level = 2	Asking and Giving Directions
If Chap = 6 and Eva_Ob = 3 and Level = 3	Asking and Giving Directions

Data representation for data transformation is as follows:

Chapter (Chap) represents 1= Greeting-Basic, 2 = Feeling and Emotion, 3 = Weather, 4 = Health, 5 = University life, 6 = Asking and Giving Directions

Observation (Eva_Ob) represents 1 = Pronunciation, 2 = Grammar, 3 = Fluency

Level Skill (Level) = Pronunciation, Grammar, and Fluency represents each Observation as follows:

Most 4, Moderate 3, Low 2, Very low 1

From Table 7, we can observe that the data used for learning to classify future data types based on the training set has generated 18 rules with interrelated relationships. These rules are described as follows:

Rule 1: Students who received training in the Greeting-Basic topic and have a pronunciation proficiency level of 4 (able to pronounce correctly) tend to have excellent communication outcomes.

Rule 2: Students who received training in the Greeting-Basic topic and have a grammar proficiency level of 3 (able to use grammar structures correctly with minor errors) tend to have good communication outcomes.

Rule 3: Students who received training in the Greeting-Basic topic and have a fluency proficiency level of 4 (speak conversationally and fluently like a native speaker) tend to have natural and continuous communication outcomes.

Rule 4: Students who received training in the Feeling and Emotion topic and have a pronunciation proficiency level of 3 (occasional pronunciation errors) tend to have communication outcomes with minor pronunciation errors.

Rule 5: Students who received training in the Feeling and Emotion topic and have a grammar proficiency level of 3 (able to use grammar structures correctly with minor errors) tend to have communication outcomes with minor grammar errors.

Rule 6: Students who received training in the Feeling and Emotion topic and have a fluency proficiency level of 4 (speak conversationally and fluently like a native speaker) tend to have natural and continuous communication outcomes.

Rule 7: Students who received training in the Weather topic and have a pronunciation proficiency level of 2 (pronunciation errors that make understanding difficult) tend to have communication outcomes with pronunciation difficulties.

Rule 8: Students who received training in the Weather topic and have a grammar proficiency level of 2 (basic grammar errors) tend to have communication outcomes with minor grammar errors.

Rule 9: Students who received training in the Weather topic and have a fluency proficiency level of 3 (speak conversationally and fluently with occasional hesitations) tend to have natural and continuous communication outcomes with occasional hesitations.

Rule 10: Students who received training in the Health topic and have a pronunciation proficiency level of 3 (occasional pronunciation errors) tend to have communication outcomes with minor pronunciation errors.

Rule 11: Students who received training in the Health topic and have a grammar proficiency level of 2 (basic grammar errors) tend to have communication outcomes with minor grammar errors.

Rule 12: Students who received training in the Health topic and have a fluency proficiency level of 4 (speak conversationally and fluently like a native speaker) tend to have natural and continuous communication outcomes.

Rule 13: Students who received training in the University Life topic and have a pronunciation proficiency level of 2 (pronunciation errors that make understanding difficult) tend to have communication outcomes with pronunciation difficulties.

Rule 14: Students who received training in the University Life topic and have a grammar proficiency level of 2 (basic grammar errors) tend to have communication outcomes with minor grammar errors.

Rule 15: Students who received training in the University Life topic and have a fluency proficiency level of 2 (struggle to speak and attempt to restart at times) tend to have communication outcomes with efforts to restart and some hesitations.

Rule 16: Students who received training in the Asking and Giving Directions topic and have a pronunciation proficiency level of 3 (occasional pronunciation errors) tend to have communication outcomes with pronunciation difficulties.

Rule 17: Students who received training in the Asking and Giving Directions topic and have a grammar proficiency level of 2 (basic grammar errors) tend to have communication outcomes with minor grammar errors.

Rule 18: Students who received training in the Asking and Giving Directions topic and have a fluency proficiency level of 3 (speak conversationally and fluently with occasional hesitations) tend to have natural and continuous communication outcomes with occasional hesitations.

7. Conclusions

Upon analyzing the performance before and after using the English conversation training program for communication purposes, it was discovered that the group of students enrolled in the English for Communication course during the first semester of the academic year 2022 exhibited a statistically significant difference in average scores before and after the training. The t-test resulted in a test statistic of 15.871 with 49 degrees of freedom and a significance level (sig) of .000. Consequently, it can be concluded that the training in English conversation skills for communication led to a significant improvement in English language proficiency at a significance level of .05.

As for the guidelines for adapting the English conversation training program using the random forest technique in the future, the rankings from easiest to most challenging are as follows:

Ranking 1 Greetings: This conversation topic obtained the highest scores in pronunciation, grammar, and fluency.

Ranking 2 Feeling and Emotion: This conversation topic ranked second, with a slight decrease in pronunciation scores.

Ranking 3 Health: This conversation topic ranked third, with decreases in pronunciation, grammar, and fluency scores.

Ranking 4 Asking for and Giving Directions: This conversation topic ranked fourth, with a slight decrease in scores.

Ranking 5 Weather: This conversation topic ranked fifth, with a decrease in scores.

Ranking 6 University Life: This conversation topic ranked last.

These rankings provide guidance on the relative difficulty levels of the conversation topics, which can assist in adjusting the English conversation training program effectively.

8. Discuss the Findings

The English conversation training provided in the book "StartUp" by Beatty et al. (2020) is designed to improve communication skills in personal life, studying, and working. It is suitable for learners at various proficiency levels. The results of pre- and post-training performance clearly demonstrate the positive impact of using English conversation training materials on learners' skills. This aligns with the findings of Srithongkul (2022) research, which showed that blended multimedia e-books in English language courses significantly enhance learning skills at Rajabhat Thonburi University, Samut Prakan.

From studying English conversation training that impact students' test scores using the Random Forest Technique, 18 related rules were derived. Rules 1, 3, 6, and 12, which are related at a high level (Level 4), involve the use of the "Greeting-Basic" exercise to promote pronunciation and fluency, and "Feeling and Emotion" and "Health" exercises to promote fluency. When examining the details of all three exercises, they share common characteristics with sentences used in everyday life. They contain information about health, and the dialogues are short. Furthermore, the structure and vocabulary used are at levels A1-A2 according to the Common European Framework of Reference for Languages (CEFR). This passage indicates that the study aligns with the study conducted by Pomin and Srinonyang (2020) found that the management of English language teaching in Thailand emphasizes practical language usage for daily life, aligning with a natural language learning approach. The teaching approach has shifted from a focus on grammar to an emphasis on communication skills, beginning with listening, speaking, reading, and writing in that order. As a result, students have achieved higher levels of language proficiency based on the CEFR. For the Grammar aspect, it was found that there is a proficiency level of "adequate" (Level 3) based on Rules 1, 3, 6, and 12. These rules are practiced in Greeting-Basic and Feeling and Emotion exercises. The development of English language grammar proficiency has improved from an adequate level to a good level through standardized CEFR skill training.

Additionally, analyzing the content of conversational interactions is essential for developing English speaking skills for everyday communication. This supports the ideas of Sacks, Schegloff, and Jefferson (Supakorn, 2020), who emphasize the importance of conversation analysis for understanding language usage and improving communication skills. Based on the research findings, the conversational topics were ranked according to their average scores. Greetings ranked first due to its resemblance to everyday phrases and the relatively easy structure and vocabulary of the exercises. Feeling and Emotion ranked second as students were familiar with the short dialogues from previous learning experiences. Health followed, incorporating specialized vocabulary while maintaining familiarity. Asking for and giving directions came next, focusing on providing and seeking directions, which students had limited exposure to in the lessons. Weather ranked second to last due to its longer dialogues and more specialized vocabulary. Finally, University Life received the lowest scores because of the lengthy dialogues and the variety of question-answer structures involved. It is recommended that instructors arrange the sequence of topics according to the learners' needs. According to conversational analysis theory, the organization of text sequences plays a crucial role in the effective development of learners' English speaking skills (Klanrit, 2019). Byrne (1997) also noted that teaching speaking skills requires practice in using stable components of language such as pronunciation, grammar patterns, and vocabulary, as well as opportunities for individual expression. Instructors should focus on accuracy in the initial stages and emphasize flexibility at higher levels of learning. This approach will enhance the efficiency of English communication skills in the long run.

9. Suggestion

Suggested recommendations based on the research findings are as follows:

1. The research should be replicated with a sample group studying English for Communication at Rajamangala University of Technology Tawan-ok to gather diverse research results.

2. The insights derived from the 18 correlated rules should be utilized in designing research activities aimed at addressing learning challenges in English subjects that share similar content, ensuring practical and tangible outcomes.

3. It is advisable to develop additional activities that offer students ongoing opportunities to apply English for communication in authentic real-life situations.

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References

Beatty, K., Longshaw, R. N., & Austin, H. M. (2020). *StartUp*. Hoboken: Pearson Education.

Byrne, D. (1997). *Teaching oral English* (7th ed.). Harlow: Longman.

Carroll, B. J. (1980). *Testing communicative performance*. London: Pergamon.

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). New Jersey: Lawrence Erlbaum Associates.

Denisko, D., & Hoffman, M. M. (2018). Classification and interaction in random forests. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(8), 1690-1692. doi:10.1073/pnas.1800256115

Dorfman, R. (1979). A formula for the Gini coefficient. *The Review of Economics and Statistics*, *61*, 146-149. doi:10.2307/1924845

EF English Proficiency Index. (2022). *Is English enough? But the ranking results can't tell! Thai English skills rank at the bottom of the rankings.4 Asia*. Retrieved from https://www.smartsme.co.th/content/248794

Ellis, M., & Johnson, C. (1994). *Teaching Business English*. Oxford: Oxford University Press.

Hair, J. F., Black, W. C., Babin, B. J., & Anderson, R. E. (2019). *Multivariate data analysis* (8th ed.). Hampshire: Cengage Learning.

Hengsuko, C., Boonkao, K., Srihanam, N., & Manee, W. (2019). Current situation and factors affected English language communication skill of Thai people. *MBU Education Journal: Faculty of Education Mahamakut Buddhist University*, *7*(1), 155-160.

Huynh-Cam, T. T., Chen, L. S., & Le, H. (2021). Using decision trees and random forest algorithms to predict and determine factors contributing to first-year university students' learning performance. *Algorithms*, *14*, 318. doi:10.3390/a14110318

Jayaprakash, S., Krishnan, S., & Jaiganesh, V. (2020). Predicting students academic performance using an improved random forest classifier. In *2020 International Conference on Emerging Smart Computing and Informatics (ESCI)* (pp. 238-243). Pune, India. doi:10.1109/ESCI48226.2020.9167547

Kaewpanitch, C. (2020). *Using data mining techniques to analyze most characteristics that affect the performance of undergraduate students learning English* (Master's thesis, National Institute of Development Administration, Bangkok). Retrieved from https://as.nida.ac.th/wp-content/uploads/ 2022/08/6210426022 _ชิดชนนก.pdf

Kanjanawasee, S., Pitayanon, T., & Srisukho, D. (2008). *Selection of appropriate statistics for research* (5th ed.). Bangkok: Chulalongkorn University Printing House.

Klanrit, P. (2019). Conversation analysis: An approach to teaching English speaking skill. *Faculty of Humanities and Social Sciences Review*, *7*(1), 33-51.

Madaan, M., Kumar, A., Keshri, C., Jain, R., & Nagrath, P. (2021). Loan default prediction using decision trees and random forest: A comparative study. *IOP Conference Series: Materials Science and Engineering*, *1022*, 012042. doi:10.1088/1757-899X/1022/1/012042

Meteevorakij, P., T.Chaisuwan, P., Nubpetchploy, S., & Tanwong, K. (2020). Performance measurement forecasting equations ability to study English for business communication based on the 21st century learning skills by creating decision tree. *Journal of Educational Measurement*, *37*(102), 202-218.

Pomin, T., & Srinonyang, P. (2020). Applying Web-based instruction to EFL for undergraduate students to improve the English language ability based on the reference levels of the Common European Framework of Reference for Languages. *Journal of Modern Learning Development*, *5*(5), 212-223.

Richards, J., & Rodgers, T. (2002). *Approaches and methods in language teaching* (2nd ed.). New York: Cambridge University Press.

Rujichom, K. (2018). English communication life skills of Thailand people in the 21th century. *Journal of Arts Management*, *2*(3), 199-210.

Ruksuan, P. (2022). Development of English innovation and guidebook based on communicative approach for working preparation about hospitality industry concerning hotel and accommodation services in ASEAN Community for Maehongson Community College students. *The Academic Journal: Faculty of Humanities and Social Sciences Nakhonsawan Rajabhat University*, *9*(1), 210-221.

Srithongkul, W. (2022). The development of Multimedia Electronic Book in English for Study Skills Subject of students at Dhonburi Rajabhat University Samutprakarn. *Sisaket Rajabhat University Journal*, *16*(1), 89-100.

Stansfield, C. (1973). Review of foreign language testing: Theory and practice, by John L. D. Clark. *Hispania*, *56*(3), 746-747. doi:10.2307/339288

Supakorn, S. (2020). A conversation analytic study of classroom interactional competence. *LEARN Journal: Language Education and Acquisition Research Network*, *13*(2), 15-40.

Tangpattanakit, J. (2020). Influence of factors affecting English communication with motivation as a central variable and anxiety as a controlling variable. *RMUTL Journal of Business Administration and Liberal Arts*, *8*(2), 111-126.

Thaweerat, P. (1997). *Social science research* (7th ed.). Bangkok: Charoenphon Printing.

The Office of Academic Support and Registration Rajamangala University of Technology Tawan-ok. (2005). *English for Communication Academic Year 2022*. Retrieved from http://regis.rmutto.ac.th/registrar/home.asp

Ur, P. (1996). *A course in language teaching: Practice and theory.* Cambridge: Cambridge University Press.

Wiengnil, A. (2010). *A study of out-of-class English language learning activities of secondary school students in English Program* (Master's thesis, Chulalongkorn University, Bangkok). Retrieved from http://cuir.car.chula.ac.th/bitstream/123456789/30421/1/atitaya_wi.pdf

Yates, J. (2016). *Practice makes perfect: English conversation* (2nd ed.). Tennessee: McGraw-Hill Education.

Available online at https://li02.tci-thaijo.org/index.php/ssstj

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Effect of Bioactive Peptide, KR-12-a5, on Growth of Clinical Methicillin-resistant *Staphylococcus aureus* Isolates

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Abstract

Staphylococcus aureus is one of the most frequent infectious agents causing hospitals and community associated infections. Developing the treatment for methicillin resistant *S. aureus* (MRSA) is a challenging arena for effective therapeutics. Application of antimicrobial peptides is an alternative option to control MRSA infection. The potential of the bioactive peptide against MRSA was previously revealed; however, the study on the effect of KR-12-a5 on the various clinical MRSA growth was not elucidated. This study aimed to investigate the effect of bioactive peptide KR-12-a5 on the growth of MRSA isolated from patients. Evaluation of KR-12-a5 peptide using antimicrobial susceptibility test was performed. Antimicrobial activity assay showed that the KR-12-a5 at 2-64 µM could inhibit the growth of *S. aureus* ATCC 29213 in the range of 55.0±0.8%-81.0±6.2%. In case of KR-12-a5 treatment at 2, 4, and 8 µM, growth of all twenty clinical MRSA isolates could be inhibited less than 90% in the range of 0.0-75.9±1.6%, 0.0-73.0±4.2%, and 0.0-77.1±1.7%, respectively. Whereas the treatment of those at 16, 32, and 64 µM, growth of clinical MRSA could be significantly inhibited more than 90% ($p < 0.05$) in the range of 94.3±6.7%-100.4±0.4% in 3 isolates (15%), 97.3±0.3%-99.5±0.2% in 4 isolates (20%), and 91.6±0.8%-99.0±0.1% in 9 isolates (45%), respectively. These findings indicated that the KR-12-a5 effectively controls growth of both *S. aureus* ATCC 29213 and clinical MRSA isolates.

Keywords: *Staphylococcus aureus*, MRSA, KR-12-a5, Antimicrobial peptide

1. Introduction

Staphylococcus aureus is one of the most frequent infectious causes of hospitals and communities-associated infection. The bacteria are located on the skin and mucous membranes, mostly in the nose area. Methicillin resistant *S. aureus* (MRSA) strains are divided into hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA) (Cerini et al., 2023; Otto, 2012). Developing the treatment for MRSA is challenging due to its antimicrobial resistance. Antimicrobial peptides (AMPs) are considered an alternative used to treat MRSA infection, generally made up of 10-50 amino acid residues. Antimicrobial peptides from microbes and animals are active against multidrug-resistant Gram-positive and Gram-negative bacteria especially the inhibition of biofilm formation. LL-37 is a synthetic antimicrobial peptide reveals the potential for antimicrobial activity *in vitro* against bacteria, fungi, viruses, and parasites. There are also shortening peptide lines and amino acid changes to increase the effectiveness of pathogen inhibition (Xhindoli et al., 2016). Consequently, the α-helical wheel diagram of KR-12 developed from LL-37, was altered the hydrophobicity and net positive charge of the peptides to obtain the effective short AMPs having antimicrobial activities without mammalian cell toxicity (Jacob, Park, Bang, & Shin, 2013). KR-12 was designed in a series of analogs and synthesized as KR-12-a (1-8). The antimicrobial activity of KR-12 and its analogs were determined against Gram-positive (*Bacillus subtilis*, *Staphylococcus epidermidis*, *S. aureus*), and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* Typhimurium). Studies on KR-12-a5 revealed a minimal inhibitory concentration (MIC) of 4 µM against *S. aureus* KCTC 1621 (Jacob et al., 2013) and higher (2- to 4-fold) antimicrobial activity against MRSA than LL-37 (Kim, Rajasekaran, & Shin, 2017). The activity of KR-12-a5 surpasses that of LL-37 against multidrug-resistant bacteria and demonstrates synergistic effects with antibiotics against multidrug-resistant *Pseudomonas aeruginosa* (MDRPA). Additionally, KR-12-a5 exhibits lack of cytotoxicity for mammalian cells and resistance to physiological salts, as reported by Kim et al. (2017). Consequently, KR-12-a5 emerges as a promising candidate for the development of antimicrobial agents. Even though the potential of the bioactive peptide KR-12-a5 against MRSA was previously reported (Kim et al., 2017); however, there is limited report on the effect of KR-12-a5 on growth of various MRSA isolated from patients. This study aimed to determine the effect of bioactive peptide KR-12-a5 on growth of clinical MRSA.

2. Materials and Methods

2.1 Peptide synthesis

The antimicrobial peptide KR-12-a5 (KRIVKLILKWLR-NH_2) was synthesized through standard solid-phase methods using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry (Synpeptide, China). The synthesized peptide was subsequently purified and identified utilizing HPLC and Electrospray Ionization Mass Spectrometry (ESI-MS) (Synpeptide, 2015). The purity of peptide was more than 95.5% as ascertained by HPLC. The resulting lyophilized peptide was dissolved in Millipore-purified water, yielding a stock solution with a concentration of 10 mM, and was stored at -20°C for further use.

2.2 Bacterial strains

S. aureus ATCC 29213 was used as a standard strain and the twenty clinical methicillin resistant *S. aureus* isolates in this experiment were obtained from the stock culture in a private hospital in Bangkok and Department of Microbiology, Faculty of Public Health, Mahidol University. Clinical specimens, including sputum, pus, urine, catheter tips, rectal swabs, and blood cultures, were collected during 2021-2022 and processed using standard microbiological techniques and MRSA identification was confirmed through antimicrobial susceptibility testing.

2.3 Bacterial culture

Bacteria were cultured individually on tryptic soy agar (TSA) at 37°C for 18-24 h. A single colony was picked up and inoculated into tryptic soy broth (TSB) and incubated at 37°C in 200 rpm shaker for 2-6 h. The turbidity of bacterial culture was adjusted to standardize the inoculum density for an antimicrobial susceptibility test using a $BaSO_4$ 0.5 McFarland standard (CLSI, 2018).

2.4 Evaluation of antimicrobial activity of KR-12-a5 peptide using antimicrobial susceptibility test

In the laboratory experiment, an evaluation of the antimicrobial susceptibility of KR-12-a5 was conducted. To ensure standardized conditions, the bacterial suspension used in this evaluation was adjusted to an approximate concentration of 1 x 10^8 CFU/ml. This adjustment was achieved by matching the turbidity of the bacterial suspension to that of a 0.5 McFarland standard, following the guidelines outlined in the Clinical and Laboratory Standards Institute (CLSI) document of 2018. Subsequently, the adjusted bacterial suspension was further diluted to a 1:100 ratio using a 1% peptone solution. The antimicrobial evaluation of KR-12-a5 peptide was conducted utilizing a growth rate curve analysis. This experiment took place in a controlled environment within a sterile 96-well microtiter plate. Growth rate curves allowed for a comprehensive assessment of the antibacterial activity of the peptide.

The KR-12-a5 stock peptide was two-fold diluted (4-128 µM) in 1% peptone. The 50 µl of stock peptide (2-64 µM: final concentration) and the 50 µl of diluted bacteria (~ 5x10^5 CFU/ml: final concentration) were inoculated to each well of microtiter plate (CLSI, 2018; Kim et al., 2017). The mixtures were incubated at 37°C and measured by microtiter plate reader at 600 nm every h for 18 h. The 100 µl of peptone served as a control. The 50 µl of vancomycin (2 mg/ml) combined with 50 µl of diluted bacteria (~5x10^5 CFU/ml), and 50 µl of TSB mixed with 50 µl of diluted bacteria (~5x10^5 CFU/ml) used as the positive control and negative control, respectively. To assess the inhibition percentages of KR-12-a5 and vancomycin, all experiments were done in triplicate, and the following formula was employed:

$$\% \text{ inhibition} = [(OD_{negative\ control}-OD_{treatment})/OD_{negative\ control}] \times 100\%$$

2.5 Statistical analysis

Each experiment was performed triplicate and the results were expressed as mean±SD. Analysis of variance (one-way ANOVA; assume α at 0.05) was used to analyze statistical significance of differences between the experiment groups and control groups in antimicrobial susceptibility test. The analysis was performed by using SPSS statistics 18.

3. Results and Discussion

Antimicrobial activity on growth of *S. aureus* ATCC 29213 and twenty clinical MRSA isolates was evaluated using antimicrobial susceptibility test. Bacterial culture was tested with three different treatments for 18 h incubation in 96-well plate. 1) six concentrations of KR-12-a5 (2 μM, 4 μM, 8 μM, 16 μM, 32 μM, and 64 μM), 2) TSB (negative control), and 3) antibiotic (positive control). The antimicrobial activity of all six concentrations of KR-12-a5 on *S. aureus* ATCC 29213 growth was observed within the incubation period spanning 8 to 18 h in the course of the study (Figure 1A). The results indicated that all six concentrations of KR-12-a5 exhibited significant antimicrobial effects on the growth of *S. aureus* ATCC 29213. At 18 h of experiment, those treated with all six concentrations of KR-12-a5 revealed significant differences compared to negative control (p-value < 0.01) and positive control (p-value < 0.01). The experiment depicted in Table 1 and Figure 2 suggested that KR-12-a5 peptide has an effect in controlling the growth of *S. aureus* ATCC 29213. The growth of those could be inhibited by KR-12-a5 at different concentrations. At concentrations of 2 μM, 4 μM, 8 μM, 16 μM, 32 μM, and 64 μM, the KR-12-a5 peptide demonstrated inhibitory effects on *S. aureus* ATCC 29213 growth, yielding inhibitions of 56.6±0.9%, 55.7±1.5%, 55.8±0.9%, 55.0±0.8%, 57.0±2.4%, and 81.0±6.2%, respectively. These results were consistent with antimicrobial activity against *S. aureus* KCTC 1621 with the MIC of 4 μM (Jacob et al., 2013; Kim et al., 2017).

In this investigation, twenty samples that were isolated from the various clinical samples were employed, including sputum 7 (35.0%), pus 5 (25.0%), urine 5 (25.0%), catheter tip 1 (5.0%), rectal swab 1 (5.0%), and haemoculture 1 (5.0%) isolates (Table 2). The growth of twenty clinical MRSA isolates was assessed to determine the antimicrobial action of KR-12-a5 at concentrations ranging from 2 μM to 64 μM (Figure 1B-1U and Table 1). During the time period 3-18 h, growth of all clinical MRSA isolates exhibited significant difference of least one pair (p-value < 0.05). At 18 h for the treatment of 2 μM KR-12-a5, the growth of twenty clinical MRSA isolates could be inhibited less than 90% in the range of 0.0±0.0% to 75.9±1.6%. For the treatment of 4 μM KR-12-a5, the growth of the twenty isolates were inhibited in the range of 0.0±0.0% to 73.0±4.2%. For the treatment of 8 μM KR-12-a5, the growth of the twenty isolates were inhibited in the range of 0.0±0.0% to 77.1±1.7%. For the treatment of 16 μM KR-12-a5, growth of three clinical MRSA isolates (15%) including isolate 8, isolate 13, and isolate 16, were inhibited more than 90% within 18 h in the range of 94.3±6.7% to 100.4±0.4%, whereas the growth of other 17 clinical MRSA isolates were inhibited less than 90% in the range of 28.1±9.4% to 79.9±3.3%. For the treatment of 32 μM KR-12-a5, growth of four clinical MRSA isolates (20%) (isolate 2, isolate 8, isolate 13, and isolate 16) were inhibited more than 90% in the range of 97.3±0.3% to 99.5±0.2%, whereas the growth of other 16 clinical MRSA isolates were inhibited less than 90% in the range of 20.1±7.8% to 88.8±1.6%. For the treatment at 64 μM, growth of nine clinical MRSA isolates (45%) (isolate 1, isolate 2, isolate 3, isolate 4, isolate 7, isolate 8, isolate 9, isolate 13, and isolate 16) were inhibited more than 90% in the range of 91.6±0.8% to 99.0±0.1%, whereas the growth of other 11 clinical MRSA isolates were inhibited less than 90% in the range of 66.4±5.6% to 85.4±0.8% (Table 1 and Figure 2).

The percent inhibition resulting from the 64 μM KR-12-a5 treatment was also compared at 6, 12, and 18 h, as illustrated in Figure 2. The growth of *S. aureus* ATCC 29213 was inhibited for 96.6±1.0%, 94.1±4.4%, and 81.0±6.2%, respectively, and those of twenty clinical MRSA isolates were inhibited in the range of 68.4±6.0% to 100.4±0.7%, 83.4±0.9% to 98.4±0.6%, and 66.4±5.6% to 99.0±0.1%, respectively. Despite the apparent inhibitions observed during the early stages of growth in both *S. aureus* ATCC 29213 and twenty clinical MRSA

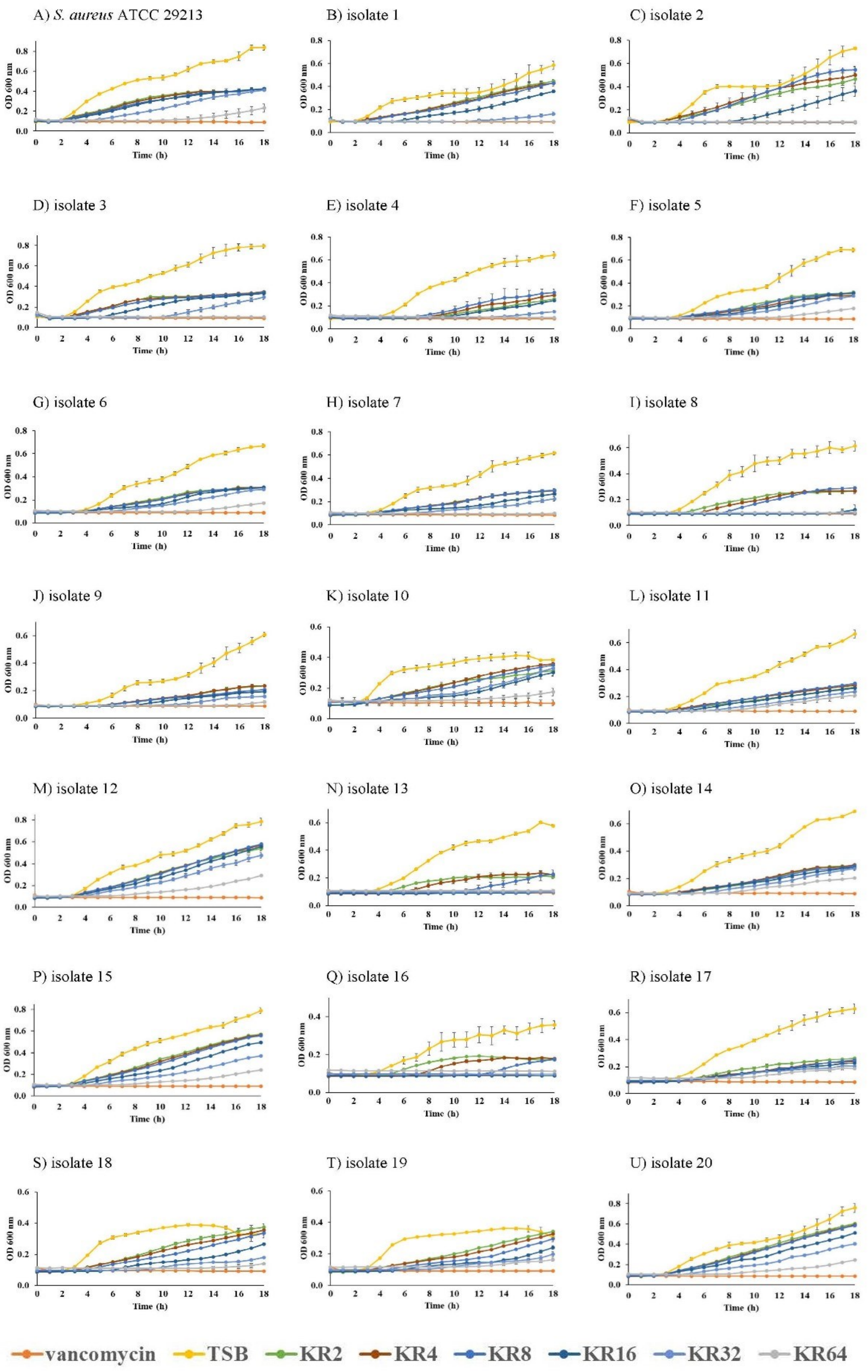


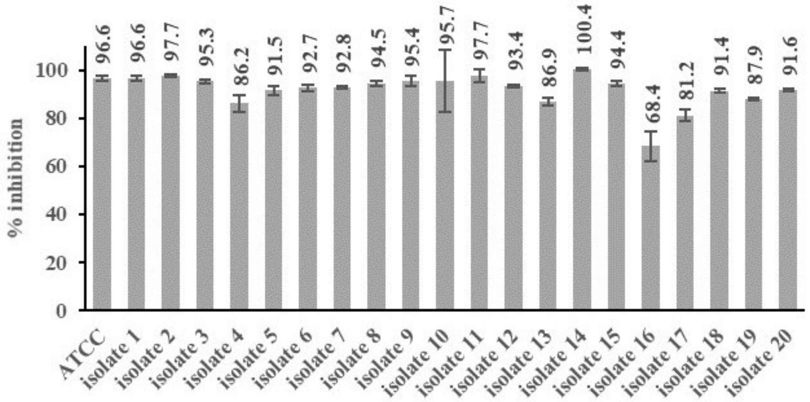
Figure 1. Antimicrobial activity of 2 - 64 µM KR-12-a5 on growth of *S. aureus* ATCC 29213 A) and clinical MRSA strains, B) - U) isolate 1-20 comparison to positive control (vancomycin) and negative control (TSB). All experiments were done in triplicate.

Table 1. Inhibitory effect and percent inhibition of KR-12-a5 on growth of *S. aureus* ATCC 29213 and clinical MRSA isolate 1-20 at 18 h of treatment.

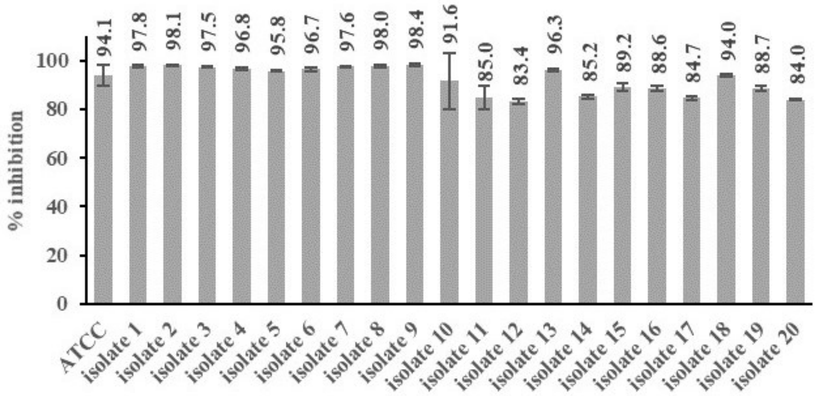
Stains	Concentrations (µM)					
	2	4	8	16	32	64
S. aureus ATCC 29213	+ 56.6±0.9%	+ 55.7±1.5%	+ 55.8±0.9%	+ 55.0±0.8%	+ 57.0±2.4%	+ 81.0±6.2%
Isolate 1	+ 27.7±6.0%	+ 31.1±9.3%	+ 30.9±3.0%	+ 46.2±1.3%	+ 85.6±2.5%	++ 98.7±0.4%
Isolate 2	+ 41.6±4.1%	+ 35.9±12.7%	+ 29.2±3.2%	+ 57.4±7.4%	++ 99.5±0.2%	++ 99.0±0.1%
Isolate 3	+ 64.1±1.6%	+ 63.2±0.2%	+ 64.3±0.7%	+ 65.5±1.5%	+ 70.7±3.2%	++ 98.0±0.2%
Isolate 4	+ 69.3±2.6%	+ 63.2±3.1%	+ 58.9±7.9%	+ 71.4±2.2%	+ 88.8±1.6%	++ 98.1±0.3%
Isolate 5	+ 61.1±0.8%	+ 65.9±1.1%	+ 62.6±1.2%	+ 62.7±2.0%	+ 66.5±2.3%	+ 84.5±1.2%
Isolate 6	+ 62.5±0.5%	+ 62.1±0.7%	+ 62.4±1.3%	+ 62.2±1.0%	+ 64.7±1.0%	+ 85.4±0.8%
Isolate 7	+ 60.9±2.0%	+ 60.8±2.0%	+ 60.0±1.5%	+ 65.8±1.9%	+ 74.2±3.1%	++ 97.8±1.3%
Isolate 8	+ 66.3±1.0%	+ 66.3±2.2%	+ 61.8±1.0%	++ 94.3±6.7%	++ 98.9±0.4%	++ 97.5±0.0%
Isolate 9	+ 71.8±2.8%	+ 72.0±0.6%	+ 77.1±1.7%	+ 79.9±3.3%	+ 86.5±0.8%	++ 94.3±2.2%
Isolate 10	+ 25.6±14.5%	+ 9.5±4.3%	+ 12.4±3.9%	+ 29.7±12.0%	+ 20.1±7.8%	+ 75.0±11.2%
Isolate 11	+ 68.4±1.6%	+ 66.0±0.6%	+ 64.0±2.3%	+ 70.0±1.1%	+ 74.5±0.6%	+ 79.2±2.2%
Isolate 12	+ 35.6±4.3%	+ 31.0±2.8%	+ 29.6±3.5%	+ 33.1±3.5%	+ 44.6±2.1%	+ 70.9±1.3%
Isolate 13	+ 75.9±1.6%	+ 73.0±4.2%	+ 72.3±6.8%	++ 97.2±2.6%	++ 99.2±0.3%	++ 97.0±0.2%
Isolate 14	+ 65.7±1.1%	+ 65.2±1.6%	+ 66.3±2.4%	+ 68.0±2.6%	+ 69.6±3.1%	+ 81.2±1.0%
Isolate 15	+ 31.1±3.4%	+ 32.1±2.7%	+ 33.0±3.4%	+ 42.0±1.6%	+ 59.7±2.5%	+ 78.2±0.8%
Isolate 16	+ 68.9±2.8%	+ 66.8±2.1%	+ 67.8±2.2%	++ 100.4±0.4%	++ 97.3±0.3%	++ 91.6±0.8%
Isolate 17	+ 67.5±2.1%	+ 71.3±4.3%	+ 70.1±3.3%	+ 73.9±0.8%	+ 78.0±2.7%	+ 81.3±2.3%
Isolate 18	- 0.0±0.0%	- 0.0±0.0%	- 0.0±0.0%	+ 28.1±9.4%	+ 63.5±5.1%	+ 80.6±5.1%
Isolate 19	- 0.0±0.0%	- 0.0±0.0%	+ 3.9±12.6%	+ 29.2±16.4%	+ 50.2±9.9%	+ 66.4±5.6%
Isolate 20	+ 22.6±5.6%	+ 24.8±3.6%	+ 25.5±3.0%	+ 36.3±3.8%	+ 52.4±3.8%	+ 76.2±0.2%

- means no inhibition; + means % inhibition less than 90% and ++ means % inhibition more than 90%

A)



B)



C)

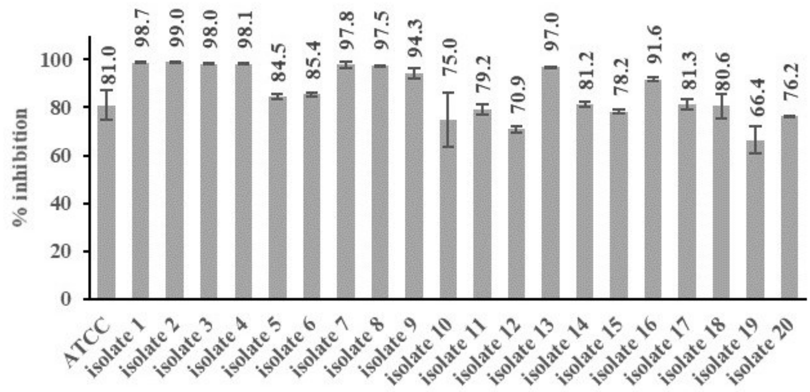


Figure 2. Percent inhibition of 64 µM KR-12-a5 on growth of *S. aureus* ATCC 29213 and clinical MRSA isolate 1-20 at A) 6 h, B) 12 h, and C) 18 h of treatment. All experiments were done in triplicate.

Table 2. Source of clinical MRSA in stock culture

Isolates	Specimen	Diseases in patient
1	Sputum	Acute respiratory failure
2	Pus	Acute bronchitis
3	Sputum	Cancer
4	Pus	Pneumonia
5	Urine	Cancer
6	Pus	Cancer
7	Catheter tip	Cancer
8	Rectal swab	Cancer
9	Pus	Pneumonia
10	Sputum	Liver cirrhosis
11	Pus	Osteomyelitis
12	Sputum	Cancer
13	Urine	Cancer
14	Urine	Cancer
15	Urine	Cancer
16	Sputum	Cancer
17	Urine	Cancer
18	Sputum	Osteomyelitis
19	Hemoculture	Cellulitis
20	Sputum	Cancer

isolates, the antibacterial activity at 6 and 12 h of treatment exhibited a consistent trend, aligning with the observations made at 18 h.

The growth anomalies were observed in some isolates. From the unexpected growth pattern especially early decline phase in isolate 10, isolate 18, and isolate 19, the growth of those isolates was in the death phase after 16 h. Despite all of them being MRSA and having received similar KR-12-a5 treatment, the divergent outcomes could be attributed to several factors, including variations in the source of clinical MRSA, the presence of distinct drug-resistant strains, and disparities in antimicrobial susceptibility patterns. These multifaceted elements contribute to the heterogeneous response to treatment, emphasizing the complexity of MRSA infections and highlighting the need for comprehensive research to elucidate the underlying mechanisms governing treatment efficacy in diverse clinical scenarios.

The effect of antimicrobial peptide OP-145 on growth of ten clinical MRSA isolates has been determined (Ming & Huang, 2017). The OP-145 showed significant antibacterial activity against nine clinical MRSA strains (90%, p-value < 0.05). Previous research on the effect of KR-12-a5 on growth of MRSA isolated from patients is very limited. The present study showed that treatment with KR-12-a5 at concentrations ranging from 4 µM to 8 μM for 18 h resulted in bacterial inhibition for a subset of MRSA isolates. Specifically, out of the total isolates tested, twelve isolates (60%) showed bacterial inhibition in the range of 60.8±2.0% to 73.0±4.2%, while eleven isolates (55%) exhibited inhibition in the range of 60.0±1.5% to 77.1±1.7%. These findings suggested that the treatment with KR-12-a5 at concentrations between 4 μM and 8 μM were successful in inhibiting the growth of MRSA isolates. Furthermore, the results from our study showed that the antimicrobial activity of KR-12-a5 was consistent with three MRSA strains, specifically CCARM 3089, CCARM 3090, and CCARM 3095 with the MIC in the range of 4 μM to 8 μM (Jacob et al., 2013; Kim et al., 2017).

Besides, KR-12-a5 exhibited an HC_{50} value of 96 μM and did not cause any hemolytic activity at MIC 4 μM (Jacob et al., 2013). Previous study revealed that KR-12-a5 is a non-cytotoxic agent with potent antimicrobial and antibiofilm activity against pathogens; moreover, KR-12-a5 might serve as a potential therapeutic agent and play key effects in the treatment of infections (Caiaffa et al., 2017; Li et al., 2019). Limitations in this study were the lack of MIC determination of each MRSA isolate and the presence of early death phase in some isolates.

4. Conclusions

Antimicrobial activity assay demonstrated that the KR-12-a5 at concentrations ranging from 2 μM to 64 μM inhibited the growth of *S. aureus* ATCC 29213 at 18 h with the growth inhibition percentages ranged from 55.0±0.8% to 81.0±6.2%. The antimicrobial activity of KR-12-a5 against twenty clinical MRSA isolates was determined. In this study, the KR-12-a5 inhibited growth of all tested clinical MRSA isolates less than 90% at the concentrations of 2 μM, 4 μM, and 8 μM in ranged of 0.0±0.0%-75.9±1.6%, 0.0±0.0%-73.0±4.2%, and 0.0±0.0%-77.1±1.7%, respectively. Nevertheless, KR-12-a5 at the concentration of 16 μM, 32 μM, and 64 μM significantly inhibited growth of clinical MRSA isolates more than 90% ($p < 0.05$) in 3 isolates (15%), 4 isolates (20%), and 9 isolates (45%) in ranged of 94.3±6.7%-100.4±0.4%, 97.3±0.3%-99.5±0.2% and 91.6±0.8%-99.0±0.1%, respectively. These findings indicate that bioactive peptide KR-12-a5 effectively inhibits growth of both *S. aureus* ATCC 29213 and clinical MRSA isolates. The review of KR-12-a5 peptide on this research is rarely carried out, especially when MRSA that is isolated from patients.

This pioneering research delved into MRSA strains collected from patients in Thailand. Remarkably, despite uniform treatment, diverse outcomes emerged, highlighting the distinct characteristics of the studied MRSA variants. This study provides valuable data for the practical application of the investigated peptide, offering insights into its potential as a therapeutic or preservative agent. For further studies, the determination of MIC and MBC is pivotal in evaluating the safety and efficacy of antimicrobial peptide. The findings contribute to the growing body of knowledge surrounding antimicrobial peptides, paving the way for their judicious utilization in various fields.

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Conflict of Interest

No conflict of interest.

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Ethical Approval

This research was reviewed and approved according to the Standard Operating Procedures of Ethical Review Committee for Human Research, Faculty of Public Health, Mahidol University, to consider to be complied with a "Research with Exemption" category (Protocol number 40/2564).

Publication Ethic

The submitted manuscript has not been previously published or is under review for publication by another print or online journal or source.

References

Caiaffa, K. S., Massunari, L., Danelon, M., Abuna, G. F., Bedran, T. B. L., Santos-Filho, N. A., … Duque, C. (2017). KR-12-a5 is a non-cytotoxic agent with potent antimicrobial effects against oral pathogens. *Biofouling*, *33*(10), 807-818. doi:10.1080/08927014.2017.1370087

Cerini, P., Meduri, F. R., Tomassetti, F., Polidori, I., Brugneti, M., Nicolai, E., … Broccolo, F. (2023). Trends in antibiotic resistance of nosocomial and community-acquired infections in Italy. *Antibiotics*, *12*(4), 651. doi:10.3390/antibiotics12040651

CLSI. (2018). *M07 Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically* (11th ed.). Pennsylvania: Clinical and Laboratory Standards Institute.

Jacob, B., Park, I. S., Bang, J. K., & Shin, S. Y. (2013). Short KR-12 analogs designed from human cathelicidin LL-37 possessing both antimicrobial and antiendotoxic activities without mammalian cell toxicity. *Journal of Peptide Science*, *19*(11), 700-707. doi:10.1002/psc.2552

Kim, E. Y., Rajasekaran, G., & Shin, S. Y. (2017). LL-37-derived short antimicrobial peptide KR-12-a5 and its D-amino acid substituted analogs with cell selectivity, anti-biofilm activity, synergistic effect with conventional antibiotics, and anti-inflammatory activity. *European Journal of Medicinal Chemistry*, *136*, 428-441. doi:10.1016/j.ejmech.2017.05.028

Li, H., Zhang, S., Nie, B., Long, T., Qu, X., & Yue, B. (2019). KR-12-a5 reverses adverse effects of lipopolysaccharides on HBMSC osteogenic differentiation by influencing BMP/Smad and P38 MAPK signaling pathways. *Frontiers in Pharmacology*, *10*, 639. doi:10.3389/fphar.2019.00639

Ming, L., & Huang, J. A. (2017). The antibacterial effects of antimicrobial peptides OP-145 against clinically isolated multi-resistant strains. *Japanese Journal of Infectious Diseases*, *70*(6), 601-603. doi:10.7883/yoken.JJID.2017.090

Otto, M. (2012). MRSA virulence and spread. *Cellular Microbiology*, *14*(10), 1513-1521. doi:10.1111/j.1462-5822.2012.01832.x

Synpeptide. (2015). *Custom peptide synthesis*. Retrieved from http://www.synpeptide.com/custom.asp?id=15

Xhindoli, D., Pacor, S., Benincasa, M., Scocchi, M., Gennaro, R., & Tossi, A. (2016). The human cathelicidin LL-37-a pore-forming antibacterial peptide and host-cell modulator. *Biochimica et Biophysica Acta - Biomembranes*, *1858*(3), 546-566. doi:10.1016/j.bbamem.2015.11.003

Available online at https://li02.tci-thaijo.org/index.php/ssstj

Suan Sunandha Science and Technology Journal

Microbiological Assessment of Car Doors and Steering Wheels at Benue State University, Makurdi: Public Health Implications

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Abstract

Microbiological assessments of car surfaces remain a fundamental approach to control hotspots of microbial contamination. This study was aimed at assessing the level microbial contaminations associated with car doors and steering wheel of cars within the faculty of science, Benue state university, Makurdi. A total of forty (40) samples were collected in duplicates. These included twenty duplicate samples from car door handles and twenty duplicate samples from car steering wheels respectively using sterile swab sticks and transported to Charis Research and Diagnostic laboratory for analysis. The samples were analysed using cultural, biochemical and morphological techniques. The results revealed that the heterotrophic bacterial count range from 1.97 x 10^4 to 2.41 x 10^4 CFU/cm^2 while the fungi count range from 1.9 x 10^3 to 3.7 x 10^3 CFU/cm^2. *Staphylococcus* spp. had the highest occurrence of 14(70%) and 9(45%), *Proteus* spp. had an occurrence of 6(30%) and 2(10%) for car door handles and car steering wheels while there was no detection of *Salmonella* in all the samples assessed. The fungi occurrence rate observed was *Aspergillus* spp. [7(35%)] for car door handle and 3(15%) for car steering wheel while *Rhizopus* spp. had a prevalence rate of 4(20%) for car door handle and 1(5%) for car steering wheel. This study affirmed that car surfaces could serve as a reservoir of potential pathogens. Hence, routine disinfection of these surfaces is very important.

Keywords: Microbiological assessment, Car doors, Steering wheels, Nigeria

1. Introduction

Human microbiome is influenced by several factors including environmental exposure to microorganisms encountered via physical contacts (Stephenson, Gutierrez, Peters, Nichols, & Boles, 2014). Several microorganisms can survive on inanimate objects long enough to be transmitted within human population having contact with the contaminated surfaces (Osei, Nyarko, & Atter, 2021). Microbiological investigation of frequently touched surfaces has become an interest to researchers because contaminated surfaces could serve as reservoir of potential pathogens (Maori, Agbor, & Ahmed, 2013; Nworie, Ayeni, Eze, & Azi, 2012). Public door handles are often hotspots for microbial contamination especially because of the frequent and inevitable use of most door handles by different individual (Ikede Rex et al., 2022).

The major source of the spread of community acquired infections are fomites; such fomites include door handles of convenience, showers toilet seats and faucets, sinks lockers, chairs, and tables especially those found in public places such as markets, banks, dormitories, schools, churches, public offices, hospitals, hotels, restaurants and rest rooms (Bright, Boone, & Gerba, 2010). The handle of car doors and steering wheel are one of the most important reservoirs of potential pathogens that human have direct contact with on daily basis (Al-Harmoosh, Eidan, Al-Hadrawy, Mohammed, & Hamed, 2018). Previous investigations have reported that commonly used surfaces such as door handles, car steering, desk and other items can be a major source of potentially pathogenic microbial contaminants (Nwankwo, Okey-kalu, & Eze, 2022, Stephenson et al., 2014).

Several microorganisms have been associated with public surfaces including *Staphylococcus*, *Micrococcus,* Enterobacteriaceae and several fungi genera (Al-Ghamdi et al., 2011; Oluyemi, Oluyemi, & Omonike, 2018; Zenbaba et al., 2023). *Staphylococcus* species are notably a commensal microorganism that colonizes the human population but could simultaneously trigger opportunistic infections of soft tissues, skin, blood, septic arthritis, sepsis and pneumonia (Kozajda, Jeżak, & Kapsa, 2019; Nadimpalli et al., 2018). Similarly, *Proteus* spp. of the Enterobacteriaceae family is also usually classified a natural microflora of the gut and skin (Drzewiecka, 2016) but has been implicated as potential pathogen associated with Crohn's disease (Hamilton, Kamm, Ng, & Morrison, 2018). Fungal-linked infections are relatively not frequent in immunocompetent and healthy individuals like the bacterial infections, although some ubiquitous fungal can still cause lethal diseases in immunocompromised individuals (Gnat, Łagowski, Nowakiewicz, & Dylag, 2021). Some common invasive infection-inducible fungal include the *Aspergillus* species, *Candida* species, *Pneumocystis* species and *Cryptococcus* species (Fang et al., 2023). Exposure of food materials to unclean hand surfaces colonized with these groups of microorganisms could influence food contamination (Nworie et al., 2012). Foodborne diseases are a widespread and growing public health concern globally because of the health hazards they constitute. Benue State University, Makurdi is an institution situated in the capital city of Benue state and also abode a large population of individuals being the only state-owned university in Benue state, Nigeria. Hence, the study aimed at assessing the microorganisms associated with car doors and steering wheel of cars at Benue State University, Makurdi.

2. Materials and Methods

2.1 Study area

This is a cross-sectional study carried out in the faculty of science, Benue State University, Makurdi. The investigation was conducted across four departments which include biology, chemistry, physics and maths/computer department. Benue state university is a state-owned university situated in Makurdi, Benue state, Nigeria.

2.2 Bacterial and fungal isolation

A total of twenty car door handles and twenty steering wheels swab samples were collected randomly across departments at the early hours of the day and investigated in this study using sterile swab sticks that were pre-moistened using sterile normal saline water based on ISO/DIS 14698-1 recommendation (International Organization for Standardization [ISO], 2003), then transported to Charis Rhema Research and Diagnostic Laboratory immediately on ice-packs for microbiological analysis in accordance with the protocols of Cheesbrough (2006). The samples were collected by swabbing the surfaces of car door handles and steering wheels with properly labeled sterile swab sticks aseptically. The swab samples were soaked in test tubes containing 9mL of normal saline and serially diluted to get diluent 10^1, 10^2, 10^3, 10^4 and 10^5 respectively. Using serial dilution techniques, 100 µL of diluents was inoculated into Nutrient Agar (NA) (Lab M, Lancashire, United Kingdom) plates in triplicates for heterotrophic bacteria enumeration, Eosin methylene blue agar (EMBA) (HiMedia) for enumeration of *Proteus* spp., Mannitol salt agar (MSA) (HiMedia) for enumeration of *Staphylococcus* spp. and Xylose lysine deoxycholate agar (XLDA) (HiMedia) for enumeration of *Salmonella* spp. then incubated at 37ºC for 24 hours. The detection of fungal spores was investigated using pour plate method on Potato dextrose agar (HiMedia) and incubated at 25±2ºC for 5-7 days. After incubation, the agar culture plates were observed and recorded. Based on phenotypic characterization, distinct colorless and transparent colonies on EMBA are to be considered as presumptive *Proteus* spp. isolates, distinct yellow colonies on MSA are to be considered as presumptive *Staphylococcus* spp. isolates, distinct black colonies on XLDA are to be considered as presumptive *Salmonella* spp. The presumptive isolates were purified on nutrient agar and subsequently stored on nutrient agar slants at 4ºC for further analysis.

2.3 Morphological and biochemical characterization of bacterial and fungal isolates

The characteristic growth of the isolates obtained from EMBA, MSA and XLDA medium were further characterized by Gram staining, motility test, coagulase test, indole test, catalase test, oxidase test, hydrogen sulphide test, urease test and sugar fermentation test. The cultural characteristics of fungi isolates indicated the presence of *Rhizopus* spp. with white cottony mycelia with black dots. The presence of *Aspergillus* spp. was characterized by white to brown/black colonial characteristics and conidiospores arising from a foot cell with conidia produced in chains. Microscopic identification of fungal isolates was carried out using wet mount preparation which was examined using x40 objective lens. There is the production of sporangiospores in a spherical sporangium.

2.4 Data analysis

All data in this study were analysed using the statistical package (SPSS) version 21.0 and Microsoft Excel 2013. Descriptive statistics were used to analyze the enumeration of the respective microorganisms.

3. Results

The result of this study shows that the total microbial load ranges between 1.97×10^4 - 2.41×10^4 CFU/cm^2 for bacteria and 1.9×10^3 - 3.7×10^3 CFU/cm^2 for fungi. This study also reveals that the total fungi count from the car steering and car door handle of cars in the department of biology, physics, chemistry and mathematics/computer were higher with 3.7×10^3, 2.9×10^3, 3.4×10^3 and 1.9×10^3 CFU/cm^2 respectively as compared to the total bacterial count with 2.1×10^4, 1.9×10^4, 2.4×10^4 and 2.2×10^4 CFU/cm^2 for biology, physics, chemistry and mathematics/computer department.

Table 1. Total bacteria and fungi count.

Location	Number of Samples	TBC (CFU/cm^2 x 10^4)	TFC (CFU/cm^2 x 10^3)
Biology Dept.	10	2.11±1.02	3.7±2.05
Physics Dept.	10	1.97±0.14	2.9±1.31
Maths/Computer Dept.	10	2.23±1.21	1.9±0.87
Chemistry Dept.	10	2.41±0.90	3.4±0.11

Key: TBC = Total Heterotrophic Bacterial Count, TFC = Total Fungi Count, Dept. = Department.

Staphylococcus spp. had the highest occurrence of 14(70%) for the car door handle and 9(45%) for car steering wheel as compared to *Proteus* spp. with 6(30%) for car door handle and 2(18.2%) for car steering wheel. Car door handle had a higher contamination rate of 20% while car steering wheel had a least contamination rate of 11%.

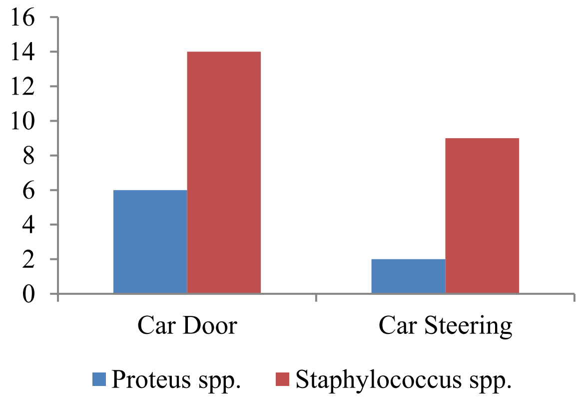

Figure 1. Occurrence of bacterial isolate of car door handle and car steering wheel.

The prevalence of fungi isolate was highest for *Aspergillus* spp. with 7(35%) for car door handle and 3(15%) for car steering wheel while *Rhizopus* spp. had a prevalence rate of 4(20%) for car door handle and 1(5%) for car steering wheel. The highest prevalence rate of fungi was recorded in physics department 6(40%). Chemistry and

Mathematics and Computer had a prevalence rate of 4(26.7%) respectively while Biology department had the least prevalence of 1(6.6%).

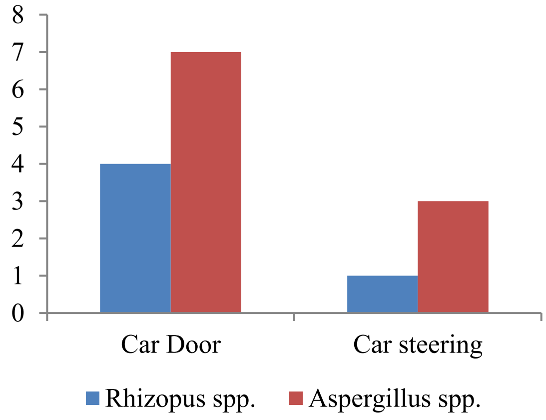

Figure 2. Occurrence of fungal isolate of car door handle and car steering wheel.

4. Discussion

Public surfaces such as door handles are very prone to microbial contamination and these surfaces are potential reservoirs of pathogens which subsequently encourage cross contamination and infection spread (Bright et al., 2010). In this study, the total microbial load range observed range from 1.97×10^4 - 2.41×10^4 CFU/cm^2 for heterotrophic bacteria and 1.9×10^3 - 3.7×10^3 CFU/cm^2 for fungi. This is lower in comparison to total heterotrophic bacterial counts range of 1.04±0.06 – 7.04±0.05 CFU/cm^2 observed on assessment of toilet door handles of schools in Nigeria. This study also reveals that the total fungi count from the car steering and car door handle in the department of biology, physics, chemistry, mathematics/computer were higher with 3.7×10^3, 2.9×10^3, 3.4×10^3 and 1.9×10^3 CFU/cm^2 respectively as compared to the total bacterial count with 2.1×10^4, 1.9×10^4, 2.4×10^4 and 2.2×10^4 CFU/cm^2 for biology, physics, chemistry and mathematics/computer department. The result of this study revealed a higher heterotrophic bacterial count across all the departments as compared to the total fungi count. In this study, the detection of *Staphylococcus* spp. and *Proteus* spp. was observed while *Salmonella* spp. was absent. Several studies have also reported the presence of *Staphylococcus* in bacteriological investigation door handles (Bashir, Muhammed, Sani, & Kawo, 2016; Ikede Rex et al., 2022). The presence of *Staphylococcus* in door handles and surface exposed to frequent human touch could be attributed to its ubiquitous presence on human skin. Al-Harmoosh et al. (2018) have also reported the detection of *Proteus* in comparable study. In contrary to this study, Al-Harmoosh et al. (2018) and Dawodu and Akanbi (2021) reported the detection of *Salmonella* in car door handles and automated teller machines respectively which was absent in this study. The disparity in rate of occurrence could be attributed differences in bacteria adaptive and physiological properties as these factors influences microbial tolerance to environmental factors. However, the ability of pathogenic strains of *Staphylococcus* species to survive unfavourable conditions for a long period of time and could still be contagious to exposed humans have been previously reported (Hübner, Hübner, Kramer, & Assadian, 2011). Furthermore, *Staphylococcus* species have also been linked to several virulence determinants (Conlon, 2014; Igbinosa, Beshiru, Akporehe, & Ogofure, 2016; Oheagbulem, Oche, Akuakolam, & Akinnibosun, 2023). Although, exposure of human to microbial contaminants such as *Staphylococcus* species via mouth or broken skin tissues could induce infections, they are usually minimal in young and healthy human population as they are capable of eliminating the pathogens within few hours or days prior to exposure (Angen et al., 2017). Some staphylococcal strains are capable of producing specific immunomodulatory toxins which may induce toxic shock syndrome (Sergelidis & Angelidis, 2017) and food poisoning (Denayer, Delbrassinne, Nia, & Botteldoorn, 2017). Similarly, several food poisoning outbreaks have been linked to *Proteus* species resulting from unhygienic food handling practices (Drzewiecka, 2016; Ma et al., 2022). *Proteus* species are associated with diverse virulence factors potentially relevant to gastrointestinal pathogenicity including the production of hemolysins, urease and IgA proteases (Hamilton et al., 2018).

The detection of *Aspergillus* spp. and *Rhizopus* spp. was observed in this study. Similar microbiological investigation has equally reported the presence of fungal spores in air microflora (Oluyemi et al., 2018). Other microbiological investigations have also reported the ubiquitous presence of fungi in other environmental samples (Akinnibosun, Beshiru, & Igbinosa, 2021; Ohagim, Ikon, Matthew, & Ohagim 2017; Onwubiko & Chinyeaka, 2015). The ubiquitous presence of these spores in air microflora could be implicated as the source of contamination in car door handles and other open surfaces. Clinical investigations have revealed that prolonged exposure to fungal spores elevates the risk diseased conditions such pneumonitis, hypersensitivity, allergic alveolitis and allergic fungal sinusitis (WHO, 2009), which could subsequently cause deterioration of kidney or/and liver especially when ingested via food (Ji et al., 2019). *Aspergillus* spp. is one of the most frequently found fungal genera in the environment and several species of this genus are producer of mycotoxin which triggers mycotoxicosis in human (Nielsen, 2003). Several other fungal genera including *Rhizopus* spp., *Mucor* spp. and *Lichtheimia* spp. have been implicated in disease conditions of individuals with metabolic disorders and immunocompromised patients (Gnat et al., 2021). Contamination of food materials by mycotoxins could promote the manifestation of chronic or acute health related problems including carcinogenic, estrogenic and immunosuppressive related health challenges as influenced by factors such as time of exposure, exposure dosage, individual health conditions, age and sex (Afsah-Hejri, Jinap, Hajeb, Radu, & Shakibazadeh, 2013; Awuchi et al., 2022).

The findings of this research revealed variation in microbial load from various car surfaces and this result is consistent with the reports of Nworie et al. (2012) and Al-Harmoosh et al. (2018). This report also shows that the car door handle had more contamination rate of 11 (55%) than the car steering wheel 4 (20%) and this contrasts with the result of Osei et al. (2021) who reported a higher contamination rate of the car steering wheel. In agreement with this study, in has been previously observed that concentration of fungal spores is usually higher in outdoor surfaces, especially in relatively cool and humid conditions (Pavan & Manjunath, 2014). Previous studies have affirmed the influence of meteorological and climate factors such as temperature and relative humidity on the proliferation and dispersion of fungal spores in the environment (Zingales, Taroncher, Martino, Ruiz, & Caloni, 2022). These reports demonstrated affirmed the reservoir capacity of car door handles and steering wheels and their contributing influence to contamination of hand surfaces. Previous studies have affirmed that human hands are major route that influence the transmission and spread of disease causing microorganisms and the surge of foodborne illnesses (Ahmednur, Esmael, & Feresa, 2022; Oranusi, Akande, & Dahunsi, 2013). Inadequate hand washing practices during food handling have been reported as a prominent cause of foodborne illnesses (Odetokun et al., 2022). Although, there have been significant rise in behavioral changes and awareness towards proper hand washing and other hygienic practices post-COVID-19 pandemic (Bizuneh, Mohammed, & Yesuf, 2022), more public health campaign including improved hand hygiene practices should continually be a routine intervention in both local and urban communities. Proper and routine hand washing practices after exposure to surfaces is therefore important in the mitigation of respiratory infections and diarrheal diseases (Teumta et al., 2019). Nevertheless, further studies that could ascertain the pathogenicity and investigation regarding specific virulence factors that might be associated with these microorganisms will give better insight on their health implication.

5. Conclusions

This study emphasized on the potentials of doors handles and other surfaces to serve as reservoir of environmental and human microflora of potentially opportunistic pathogens including *Staphylococcus* species. Thereby, making it necessary for users to get enlightened on the possible risk associated the activities of these microbial contaminants in disease transmission. Strict compliance to proper hand washing practices with clean water and detergents coupled with adherence to other standard hygienic measures could sustainably curtails the chances of cross-contamination during food handling which could subsequently triggers food borne diseases. Enhanced and more stringent biosecurity measures should be implemented in food handling to control potentially pathogenic and opportunistic pathogens. Therefore adherence to routine disinfection and cleaning of door handles, steering wheel, regular washing of hands and the use of disinfectants in controlling microbial contaminants in frequently touched surfaces is important as it will reduce the disease spread and promote public health.

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Conflict of Interest

The authors declared that there are no conflicts of interest.

References

Afsah-Hejri, L., Jinap, S., Hajeb, P., Radu, S., & Shakibazadeh, S. (2013). A review on mycotoxins in food and feed: Malaysia case study. *Comprehensive Reviews in Food Science and Food Safety*, *12*(6), 629-651. doi:10.1111/1541-4337.12029

Ahmednur, M., Esmael, M., & Feresa, F. (2022). Handwashing practice of food establishment customers, microbial quality of handwashing water, and associated factors in Ginjo Kebele, Jimma Town, Southwest Ethiopia. *Environmental Health Insights*, *16*, 1-10. doi:10.1177/11786302221144197

Akinnibosun, O., Beshiru, A., & Igbinosa, E. O. (2021). Effect of lead and cadmium on soil microbial activities. *NIPES Journal of Science and Technology Research*, *3*(2), 30-45.

Al-Ghamdi, A. K., Abdelmalek, S. M. A., Ashshi, A. M., Faidah, H., Shukri, H., & Jiman-Fatani, A. A. (2011). Bacterial contamination of computer keyboards and mice, elevator buttons and shopping carts. *African Journal of Microbiology Research*, *5*(23), 3998-4003. doi:10.5897/AJMR11.770

Al-Harmoosh, R. A., Eidan, A. J., Al-Hadrawy, H. A., Mohammed, Q. A., & Hamed, A. Q. (2018). Potential bacterial contaminants in the handles of car doors. *Journal of Pure and Applied Microbiology*, *12*(4), 2193-2198. doi:10.22207/JPAM.12.4.58

Angen, Ø., Feld, L., Larsen, J., Rostgaard, K., Skov, R., Madsen, A. M., & Larsen, A. R. (2017). Transmission of methicillin-resistant *Staphylococcus aureus* to human volunteers visiting a swine farm. *Applied and Environmental Microbiology*, *83*(23). doi:10.1128/AEM.01489-17

Awuchi, C. G., Ondari, E. N., Nwozo, S., Odongo, G. A., Eseoghene, I. J., Twinomuhwezi, H., … Okpala, C. O. R. (2022). Mycotoxins' toxicological mechanisms involving humans, livestock and their associated health concerns: A review. *Toxins*, *14*(3), 167. doi:10.3390/toxins14030167

Bashir, S. F., Muhammed, H., Sani, N. M., & Kawo, A. H. (2016). Isolation and identification of bacterial contaminants from door handles of public toilets in Federal University Dutse, Jigawa State-Nigeria. *ISOR Journal of Pharmacy and Biological Sciences*, *11*(5), 53-57.

Bizuneh, H., Mohammed, S., & Yesuf, A. (2022). COVID-19 precautionary practices and associated factors among clients visiting a tertiary hospital, Addis Ababa, Ethiopia. *PLoS One*, *17*(4), 1-12. doi:10.1371/journal.pone.0267000

Bright, K. R., Boone, S. A., & Gerba, C. P. (2010). Occurrence of bacteria and viruses on elementary classroom surfaces and the potential role of classroom hygiene in the spread of infectious diseases. *The Journal of School Nursing*, *26*(1), 33-41. doi:10.1177/1059840509354383

Cheesbrough, M. (2006). *District laboratory practice in tropical countries.* New York: Cambridge University Press.

Conlon, B. P. (2014). *Staphylococcus aureus* chronic and relapsing infections: evidence of a role for persister cells: An investigation of persister cells, their formation and their role in *S. aureus* disease. *BioEssays*, *36*(10), 991-996. doi:10.1002/bies.201400080

Dawodu, O. G., & Akanbi, R. B. (2021). Isolation and identification of microorganisms associated with automated teller machines on Federal Polytechnic Ede campus. *PLOS One*, *16*(8), e0254658. doi:10.1371/journal.pone.0254658

Denayer, S., Delbrassinne, L., Nia, Y., & Botteldoorn, N. (2017). Food-borne outbreak investigation and molecular typing: High diversity of *Staphylococcus aureus* strains and importance of toxin detection. *Toxins*, *9*(12), 407. doi:10.3390/toxins9120407

Drzewiecka, D. (2016). Significance and roles of *Proteus* spp. bacteria in natural environments. *Microbial Ecology*, *72*, 741-758. doi:10.1007/s00248-015-0720-6

Fang, W., Wu, J., Cheng, M., Zhu, X., Du, M., Chen, C., … Pan, W. (2023). Diagnosis of invasive fungal infections: Challenges and recent developments. *Journal of Biomedical Science*, *30*, 42. doi:10.1186/s12929-023-00926-2

Gnat, S., Łagowski, D., Nowakiewicz, A., & Dylag, M. (2021). A global view on fungal infections in humans and animals: Opportunistic infections and microsporidioses. *Journal of Applied Microbiology*, *131*(5), 2095-2113. doi:10.1111/jam.15032

Hamilton, A. L., Kamm, M. A., Ng, S. C., & Morrison, M. (2018). *Proteus* spp. as putative gastrointestinal pathogens. *Clinical Microbiology Reviews*, *31*(3). doi:10.1128/CMR.00085-17

Hübner, N. O., Hübner, C., Kramer, A., & Assadian, O. (2011). Survival of bacterial pathogens on paper and bacterial retrieval from paper to hands: Preliminary results. *American Journal of Nursing*, *111*(12), 30-34. doi:10.1097/01.NAJ.0000408181.37017.82

Igbinosa, E. O., Beshiru, A., Akporehe, L. U., & Ogofure, A. G. (2016). Detection of methicillin-resistant staphylococci isolated from food producing animals: A public health implication. *Veterinary Science*, *3*, 14. doi:10.3390/vetsci3030014

Ikede Rex, E., Iyevhobu, K. O., Barnabas, F. O., Ibrahim, S. M., Abinokhauno, S. O., Igbuan, E. A., & Olaitan, I. A. (2022). Bacteriological assessment of door handles and knobs at the Federal School of Medical Laboratory Technology Offices in Jos. *American Journal of Biomedical Science and Research*, *7*(5), 40-45. doi:10.34297/AJBSR.2022.17.002387

International Organization for Standardization (ISO). (2003). *Clean rooms and associated controlled environments: Biocontamination control. Part 1: General principles and methods*. Geneva, Switzerland. Retrieved from http://www.iso.org

Ji, F., He, D., Olaniran, A. O., Mokoena, M. P., Xu, J., & Shi, J. (2019). Occurrence, toxicity, production and detection of *Fusarium* mycotoxin: A review. *Food Production, Processing and Nutrition*, *1*, 6. doi:10.1186/s43014-019-0007-2

Kozajda, A. Jeżak, K., & Kapsa, A. (2019). Airborne *Staphylococcus aureus* in different environments—a review. *Environmental Science and Pollution Research*, *26*, 34741-34753. doi:10.1007/s11356-019-06557-1

Ma, W. Q., Han, Y. Y., Zhou, L., Peng, W. Q., Mao, L. Y., Yang, X., … Lei, C. W. (2022). Contamination of *Proteus mirabilis* harbouring various clinically important antimicrobial resistance genes in retail meat and aquatic products from food markets in China. *Frontiers in Microbiology*, *13*, 1086800. doi:10.3389/fmicb.2022.1086800

Maori, L., Agbor, V. O., & Ahmed, W. A. (2013). The prevalence of bacterial organisms on toilet door handles in secondary schools in Bokkos L. G. A., Jos, Plateau Sate, Nigeria. *IOSR Journal of Pharmacy and Biological Sciences*, *8*(4), 85-91. doi:10.9790/3008-0848591

Nadimpalli, M. L., Stewart, J. R., Pierce, E., Pisanic, N., Love, D. C., Hall, D., … Heaney, C. D. (2018). Face mask use and persistence of livestock-associated *Staphylococcus aureus* nasal carriage among industrial hog operation workers and household contacts, USA. *Environmental Health Perspectives*, *126*(12), 127005. doi:10.1289/EHP3453

Nielsen, K. F. (2003). Mycotoxin production by indoor molds. *Fungal Genetics and Biology*, *39*(2), 103-117. doi:10.1016/s1087-1845(03)00026-4

Nwankwo, E. O., Okey-kalu, E. U., & Eze, F. A. (2022). Bacterial contamination of door handles of commercial buses in Umuahia Metropolis Abia State. *Suan Sunandha Science and Technology Journal*, *10*(1), 54-61. doi:10.5384/ssstj.v10i1.414

Nworie, A., Ayeni, J. A., Eze, U. A., & Azi, S. O. (2012). Bacterial contamination of door handles/knobs in selected public convenience in Abuja metropolis, Nigeria: A public health threat. *Continental Journal of Medical Research*, *6*(1), 7-11.

Odetokun, I. A., Afolaranmi, Z. M., Nuhu, A. A., Borokinni, B. O., Ghali-Mohammed, I., Cisse, H., & Alhaji, N. B. (2022). Knowledge and self-reported food safety practices among meat consumers in Ilorin, Nigeria. *Dialogues in Health*, *1*, 100039. doi:10.1016/j.dialog.2022.100039

Ohagim P. I., Ikon G. M., Matthew P. C., & Ohagim, G. A. (2017). Microbiological assessment of indoor air in public toilets across selected motor parks in Owerri Metropolis, Nigeria. *Journal of Microbiology and Experimentation*, *5*(6). doi:10.15406/jmen.2017.05.00166

Oheagbulem, A. S., Oche, D. A., Akuakolam, I. M., & Akinnibosun, O. (2023). Detection of PBP2a and *PVL* genes among *Staphylococcus aureus* and their methicillin-resistant strains isolated from a hospital in Sokoto Town. *Microbes and Infectious Diseases*, *4*(4), 1210-1218. doi:10.21608/mid.2022.170686.1408

Oluyemi, O. F., Oluyemi, A. K., & Omonike, K. M. (2018). Microbiological assessment of secondary school toilets wall and door handles in Ondo, Ondo State. *International Journal of Public Health and Health Systems*, *3*(6), 123-130.

Onwubiko, N. E., & Chinyeaka, A. H. (2015). Isolation and identification of bacterial contaminants from door handles in a tertiary institution in Umuahia, Abia State, Nigeria. *Nigerian Journal of Microbiology*, *29*, 3139-3147.

Oranusi, U. S., Akande, V. A., & Dahunsi, S. O. (2013). Assessment of microbial quality and antibacterial activity of commonly used hand washes. *Journal of Biological and Chemical Research*, *30*(2), 570-580.

Osei, F. A., Nyarko, H. D., & Atter, A. (2021). Assessment of microbial contaminations associated with steering wheels and palms of commercial drivers at the University of Cape Coast's taxi rank. *Microbiology Research Journal International*, *31*(9), 52-57. doi:10.9734/MRJI/2021/v31i930345

Pavan, R., & Manjunath, K. (2014)). Qualitative analysis of indoor and outdoor airborne fungi in cowshed. *Journal of Mycology*, *2014*, 985921. doi:10.1155/2014/985921

Sergelidis, D., & Angelidis, A. S. (2017). Methicillin-resistant *Staphylococcus aureus*: A controversial food-borne pathogen. *Letters in Applied Microbiology*, *64*(6), 409-418. doi:10.1111/lam.12735

Stephenson, R. E., Gutierrez, D., Peters, C., Nichols, M., & Boles, B. R. (2014). Elucidation of bacteria found in car interiors and strategies to reduce the presence of potential pathogens. *Biofouling*, *30*(3), 337-346. doi:10.1080/08927014.2013.873418

Teumta, G. M. M., Niba, L. L., Ncheuveu, N. T., Ghumbemsita, M., Itor, P. O. B., Chongwain, P, & Navti, L. K. (2019). An institution-based assessment of students' hand washing behavior. *BioMed Research International*, *2019*, 7178645. doi:10.1155/2019/7178645

WHO. (2009). *WHO guidelines for indoor air quality: Dampness and mould*. Copenhagen, Denmark: World Health Organization.

Zenbaba, D., Sahiledengle, B., Beressa, G., Desta, F., Teferu, Z., Nugusu, F., … Chattu, V. K. Bacterial contamination of healthcare workers' mobile phones in Africa: A systematic review and meta-analysis. (2023). *Tropical Medicine and Health*, *51*, 55. doi:10.1186/s41182-023-00547-3

Zingales, V., Taroncher, M., Martino, P. A., Ruiz, M. J., & Caloni, F. (2022). Climate change and effects on molds and mycotoxins. *Toxins*, *14*, 445. doi:10.3390/toxins14070445

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