

Antimicrobial Resistance Profile of *Enterococcus* spp., *Staphylococcus Aureus*, and *Klebsiella Pneumoniae* Isolates from Two Government Hospitals in Philippines

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ABSTRACT

Antimicrobial Resistance has emerged as critical global concern especially in hospital setting. It's often referred as —silent pandemic due to lack of knowledge, its rapid spread and lack of reports. Thus, this study aimed to determine the presence, frequency of occurrence, and antimicrobial susceptibility of *Enterococcus* spp., *Staphylococcus aureus* and *Klebsiella pneumoniae* isolates from most-touched surfaces in two government hospitals-Site1 (Mindanao) and Site2 (Visayas). This study employed Quantitative and Qualitative approach. Identification of isolates was done by VITEK® 2 COMPACT System as well as the Antibiotic Susceptibility test. Findings showed that a total of 38 isolates were obtained and screened for antibiotic resistance from Mindanao (Site1) and Visayas (Site2). In Site 1, *K. pneumoniae* was the most prevalent detected bacteria and occurred in all stations. Followed by *Enterococcus* spp. and *S. aureus*. The minimal number of isolates recovered from Site 2 may reflect more regular infection control practices and lower environmental contamination. The predominance of *K. pneumoniae* in Mindanao is particularly concerning, due to its role as an opportunistic pathogen, especially among immunocompromised individuals. Most isolates were demonstrated significant resistance to commonly used antibiotics such as ampicillin, penicillin and certain cephalosporins class. Moreover, majority of the isolates has MAR Index of ≥ 0.2 values, indicating exposure to high-risk environment where antibiotics are frequently used. Lastly, Most of the isolates exhibited a wide range of antibiotics resistance patterns, with high resistance to first-line of antibiotics such as Ampicillin, Amoxicillin, Benzyl penicillin, Oxacillin, Cefalexin, Cefalotin, Ceftazidime, and Cefpodoxime. That concludes the difficulty of treatment on such antibiotic therapies. Therefore, strengthening antimicrobial stewardship programs, effective infection control and antimicrobial surveillance are strongly recommended in the two target hospitals.

Keywords: Antimicrobial Resistance, *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, MAR index

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as a pressing global health and development concern, intensified by rapid population growth, urbanization, and globalization (Acar & Rostel, 2001). The World Health Organization (WHO) has described AMR as a "silent pandemic" due to its swift global spread, low public awareness, and insufficient attention in policy and public discourse.

Southeast Asia—comprising countries such as the Philippines, Myanmar, Thailand, Cambodia, Lao PDR, Vietnam, Malaysia, and Indonesia—is considered a hotspot for both emerging infectious diseases and AMR (Zellweger *et al.*, 2017). This vulnerability stems from the region's high population density, uneven economic development, limited healthcare infrastructure, and widespread antibiotic misuse. These factors

collectively heighten the risk of AMR, posing a growing threat to public health that demands urgent and coordinated action.

In the Philippines, self-medication is a common practice, with an estimated 31–66% of the population opting to treat themselves without consulting healthcare professionals (Barber *et al.*, 2017). While this approach may offer a low-cost alternative for economically disadvantaged households, it also presents significant risks. The unsupervised use of antibiotics can disrupt the body's natural microbiome and promote the development of drug-resistant bacteria (Wipperman *et al.*, 2017).

Frequently misused antibiotics in self-medication include Ciprofloxacin, Ampicillin, Methicillin, Clindamycin, Erythromycin, and Vancomycin. These drugs are often used to treat infections caused by bacteria such as *Enterococcus* spp., *Staphylococcus aureus*, and *Klebsiella pneumoniae*. However, improper and unregulated use of these medications contributes to the evolution of resistant strains, making once-treatable infections harder—and sometimes impossible—to cure.

Hospitals, in particular, have become breeding grounds for the most dangerous antibiotic-resistant pathogens, collectively known as ESKAPE pathogens—*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. These organisms are notorious for their resistance to multiple antibiotic classes, including Extended-Spectrum Beta-Lactamase (ESBL)-producing strains, Methicillin-Resistant *Staphylococcus aureus* (MRSA), and Carbapenem-Resistant (CR) strains. According to the WHO (2020), these pathogens are now widely present in both healthcare and community settings, posing a severe threat to effective infection control.

Despite the alarming rise of AMR, many countries still lack robust surveillance systems to track its spread. Addressing this issue requires a multi-pronged approach—strengthening health policies, regulating antibiotic use, and raising public awareness are all crucial steps in preventing AMR from becoming an even more devastating global health crisis.

MATERIALS AND METHODS

Research Design

This study employed both quantitative and qualitative data collection. The quantitative data was gathered based on the Minimum Inhibitory Concentration (MIC), the Multiple Antibiotic Resistance (MAR) Index, and the frequency of occurrence of bacterial isolates in the sampling stations of every sampling site. Meanwhile, the qualitative data was collected based on the presence or absence of bacterial isolates, specifically, *Enterococcus faecium*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. Additionally, the study examined the resistance and susceptibility of these bacterial isolates to various antibiotics based on the Clinical and Laboratory Standards Institute (CLSI) breakpoints.

Study Sites

This study has two interest sampling sites, one hospital was in Mindanao (Site1) and the other hospital was in Visayas (Site 2). Every hospital, three stations were established; the Pediatric Ward, the General Ward/Medical Ward, and the Hallway. The following were identified replicates such as the most-touched surfaces (Bed and Railing, IV-stand, Door-knob, Wall, Floor etc.) and fomites such as linens and pillows. A one-time collection of data was done.

Research Ethics and Protocols

When conducting this study, ethical considerations were considered as it was dealing in hospital-based research and Laboratory. The Memorandum of Agreement (MOA) was made and secured between two target hospitals before conducting the said research for the collection of air and swab samples, photo documentations, and research activities. Travel Order was also processed for transferring the collected samples in the Antimicrobial Resistance (AMR) Laboratory and for the researchers. Furthermore, Risk Assessment and Certification was made from MSU-Marawi Institutional Biosafety Committee.

Swab and Air Sample Collection

The swab samples were collected in two sampling sites; Mindanao and Visayas. Three (3) Stations were established in each site; Pediatric Ward, General Ward/ Medical Ward and Hallway. Within each station, the following surfaces was identified for swab samples collection where applicable particularly the most-touched surfaces and fomites: Machines, Wall, Desk, Chairs and Tables, and floor, Bed/Railing, IV Stand, Doorknob, Bed Wall, and Floor.

Swab samples were collected with a sterile swab and placed in a transport tube containing Nutrient Broth, a highly nutritious liquid medium used to cultivate and isolate a wide variety of microorganisms, including bacteria and fungi, and for sterility testing. Each sterile swab kit is moistened with distilled water then swabbing over the area of each surface and simultaneously twirling the swab tip. A 10cm by 10 cm dimension was the basis and Vertically, Horizontally, Left and right Diagonal strokes will be followed for getting the average population of specimen present in each swabbing area.

Air samples was collected using one (1) settle plate in each identified station in each sampling site. Each settle plate contained a non-selective agar, which was Tryptic Soy Agar (TSA). This petri dishes are left open and placed in a non-disturbance area with a corresponding scheme of 1/1/1 (1 hour, 1 meter above the floor, and 1 meter away from walls or any major obstacles) for determining the Index of Air Contamination (Pasquarella *et al.*, 2000; Napoli *et al.*, 2012). After 18-20 hrs of incubation at a controlled temperature of 37°C, the agar plates was examined for growth and the Colony-forming units (CFU) were carefully counted as it is equal to IMA (Pasquarella *et al.*, 2000).

Identification and Antibiotic Susceptibility Test of the Bacterial Isolate

For the identification and Antibiotic Susceptibility Test of the bacterial samples, the VITEK® 2 COMPACT system is a multi-testing and automated machine will be a very convenient to this study such as getting the antibiogram profiles of *Enterococcus spp.*, *Staphylococcus aureus*, and *Klebsiella spp.* Additionally, VITEK® 2 COMPACT system was used for automated Minimum Inhibitory Concentrations (MIC) by the used of the two different VITEK Cards (GP-CARD and GN CARD).

Determination of Multi-drug Resistance (MDR)

Multi-drug Resistance is defined as non-susceptible or resistance to at least one agent in three or more antimicrobial categories (Magiarakos *et al.*, 2012). The MDR character of the isolates was therefore identified by observing the resistance pattern against the antibiotics tested. In the present study, classes of antibiotics used were: Penicillin, Penicillin w/ beta lactams, Cephalosporin, Aminoglycosides, Fluoroquinolone, Macrolides, Oxazolidinone, Glycopeptides, Tetracycline, Furans, Rifamycines, Diaminopyrimides, and Phenicol.

Determination of Multiple Antibiotic Resistance Index (MAR) of isolates and Sampling sites.

The assessment of multiple antibiotic resistance (MAR) in bacterial isolates was conducted using a quantitative experimental approach to determine the extent of resistance within bacterial population. The MAR index serves as a valuable tool for identifying antibiotic-resistant bacterial strains and tracing potential sources of antibiotic contamination in the environment. This index is calculated by dividing the number of antibiotics to which a bacterial isolate exhibits resistance by the total number of antibiotics tested against it.

$$\text{MAR Index of the isolates} = \frac{\text{Number of antibiotics to which isolate is resistant}}{\text{Total number of antibiotics tested}}$$

The MAR index of the sampling site was also calculated using the equation given by Krumperman (1983):

$$\text{MAR Index of the site} = \frac{\text{Sum of the number of antibiotics resistant incidences}}{\text{Total number of antibiotics tested}}$$

(Number of antibiotics tested x no. of isolates from the site)

A higher MAR Index suggests a greater exposure of bacteria to antibiotics, which may indicate potential contamination sources and the prevalence of antibiotic-resistant strains in a given environment (Krumperman, 1983). By analyzing MAR patterns, researchers can better understand the spread of antibiotic resistance and its implications for public health and antimicrobial stewardship (Paul *et al.*, 1997; Matyar *et al.*, 2008).

Glycerol Stocking

For glycerol stocking, a sterile microfuge tube was prepared to ensure the safe storage and long-term preservation of biological specimens. The use of a microfuge tube is essential as it minimizes the risk of contamination and maintains the integrity of the sample. To facilitate proper preservation, 0.5 mL of glycerol is added to the tube. Glycerol acts as a cryoprotectant, preventing the formation of ice crystals that could damage the cells during freezing and storage. Following this, 500 μ L of the specimen sample is carefully added to the microfuge tube, ensuring a balanced mixture of the sample and glycerol for optimal preservation. The tube will be stored at -7.76°C , a standard temperature for glycerol preservation, which effectively maintains the viability of the specimen over an extended period.

To further ensure sample integrity, sealing and labeling are performed with precision. A paraffin film is wrapped around the seal of the microfuge tube, preventing potential leakage and contamination during storage and handling. Proper labeling is crucial for sample identification and organization; therefore, a label is affixed to the tube, including the species name and its designated code. This meticulous process ensures that the preserved biological specimen remains viable for future research and experimentation.

Decontamination Process

All materials used in inoculating culture samples will undergo proper decontamination to ensure safety and prevent contamination. Reusable materials like glassware and test tubes was autoclaved at 121°C for 15–30 minutes, while work surfaces and non-autoclavable items was disinfected with 1% sodium hypochlorite or 70% ethanol. Liquid waste was treated with 1% sodium hypochlorite for 20 minutes before disposal. Washing the test tubes and Petri plates it undergone dry heat sterilization at $160\text{--}180^{\circ}\text{C}$ for 15 minutes at 100°C . These measures ensured effective decontamination and compliance with proper disposal protocols.

RESULTS AND DISCUSSIONS

A total of 38 bacterial samples were collected from the two sampling sites. In Mindanao (Site 1), all the three target species appear in Pediatric Ward and Hallway stations while in General Ward, only species *Staphylococcus aureus* was not obtained. While in Visayas (Site 2), *S. aureus* and *K. pneumoniae* was obtained in Pediatric Ward and only *Enterococcus* spp. was present in Hallway stations. There was no species obtained in the Medical Ward stations.

Table 1. Presence and Absence of *Enterococcus* spp., *Staphylococcus aureus*, and *Klebsiella pneumoniae* in the two sampling sites

Species	Amai Pakpak Medical Center			Ormoc District Hospital		
	Pediatric Ward	General Ward	Hallway	Pediatric Ward	Medical Ward	Hallway
<i>Enterococcus</i> spp.	+	+	+	-	-	+
<i>Staphylococcus aureus</i>	+	-	+	+	-	-
<i>Klebsiella pneumoniae</i>	+	+	+	+	-	-

(+) Presence, (-) Absence

The presence of these three bacterial pathogens in the hospitals is commonly responsible for serious infections infection. According to Flores-Paredes *et al.* (2021), these *Enterococcus* spp., *Staphylococcus aureus*, and *Klebsiella pneumoniae* belong to the most significant contributors to hospital-acquired infections

(HAIs) and Antimicrobial Resistance, leading to serious conditions such as bloodstream, pneumonia, urinary tract infections and surgical site infections.

Enterococcus spp., *Staphylococcus aureus* and *Klebsiella pneumoniae* were belong to the identified bacteria that are very difficult to treat due to their high ability to acquire resistance to antibiotics and are the main cause of nosocomial infections worldwide, posing threat to global public health (Bereanu *et al.*, 2024).

In Figure 5, among the four species, *Klebsiella pneumoniae* had the highest number of isolates with 22 isolates. *Enterococcus faecalis* followed with 7 isolates, while *Staphylococcus aureus* had 6 isolates and least detected was *Enterococcus faecium* with only 3 isolates suggesting lower prevalence in the two sampling hospitals.

Klebsiella pneumoniae is ubiquitous in nature and predominate nearly in anybody site (Montgomerie, 1976). Dominance of these species in Mindanao (Site 1) may implies that the mucosal surfaces of patients or watcher and environmental sources such as vegetation, soil and waters was contaminated by these species that may result to cause antimicrobial resistance to the humans (Bagley, 1985).

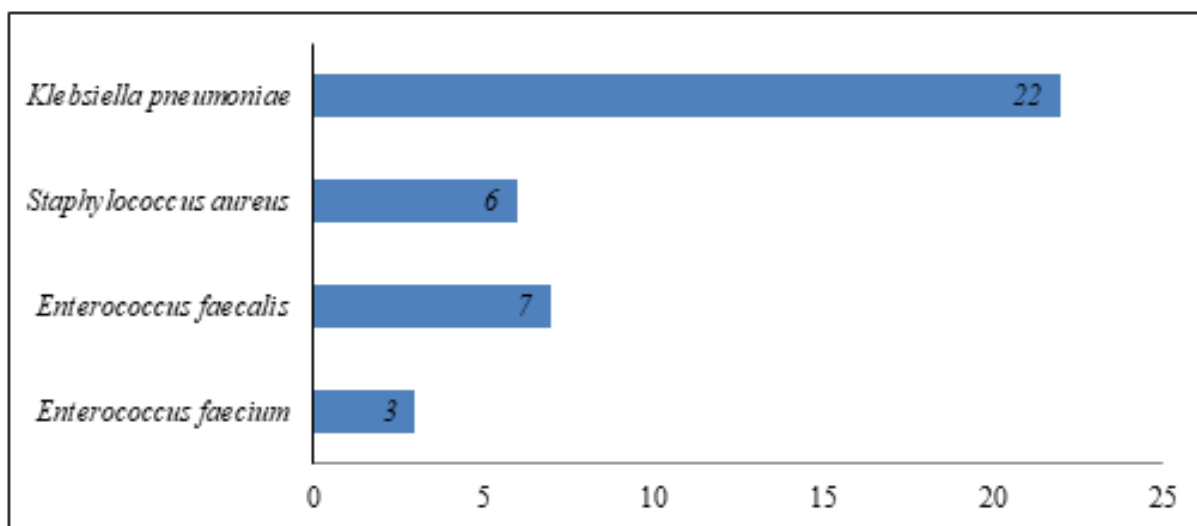


Figure 5. Prevalence of species collected from two sampling sites

The findings of Awoke *et al.* (2021) support the results of the current study, indicating that the majority of *Klebsiella pneumoniae* isolates were obtained from the pediatric ward. This is likely because *Klebsiella pneumoniae* is primarily an opportunistic pathogen that targets immune-compromised individuals, such as children and those with severe underlying conditions. Additionally, it has been identified as a cause of severe community-acquired pneumonia and is responsible for approximately one-third of all gram-negative infections.

There are many factors that play an important role in transmission of Antimicrobial resistant bacteria to humans and other animals. Hospital environment can act as a reservoir for drug-resistant pathogens (Boerlin, 2001; Hanselman, 2008). Mostly of the obtained *Klebsiella pneumoniae* was obtained in the floor maybe due to sputum or urine outside the hospital that carries by the humans (Ikeda *et al.*, 2018).

The presence of enterococci in environment especially *Enterococcus faecalis* is frequently used as faecal indicator. The *Enterococcus faecium* is less common in human gut than *E. faecalis* in hospital setting (Zhou *et al.*, 2020). Due to better adaptability of *E. faecalis* than *E. faecium*, it possess a broader range of virulence factors cause enhance its ability to cause disease (Guzman Prieto *et al.*, 2016).

Although the number of *Staphylococcus aureus* and *Enterococcus* spp. isolates are least in both hospitals, *S. aureus* remains a serious threat to public health. This is due to its resistance to commonly used first-line antibiotics, which complicates medical treatment. Humans are the major reservoir of these strains, but they have also been isolated from fomites (Neely and Maley, 2000).

Both Site 1 and Site 2, *Staphylococcus aureus* was not collected in the General Ward/ Medical ward because of several clinical, epidemiological and procedural factors. Limited or not used devices that can cause the proliferation of this pathogen (Weiner-Lastinger *et al.*, 2020) and the patient are less immunocompromised (Klevens *et al.*, 2007).

In Figure 6, shows that *Enterococcus* spp. was mostly susceptible to all the antibiotics tested except Ceftazidime (100%) and Kanamycin (100%) were resistant. Additionally, 40% of *Enterococcus* spp. isolates were resistant to Erythromycin and Quinupristin. While, All *Enterococcus* spp. identified was susceptible to antibiotics such as Cefotaxime, Gentamicin, Nitrofurantoin, Levofloxacin, Vancomycin and Tigecycline.

The results of this study show that *Enterococcus* spp. was Multi-drug resistant to different classes of antibiotics such as cephalosporin, aminoglycosides, macrolides and tetracyclines (Figure 7).

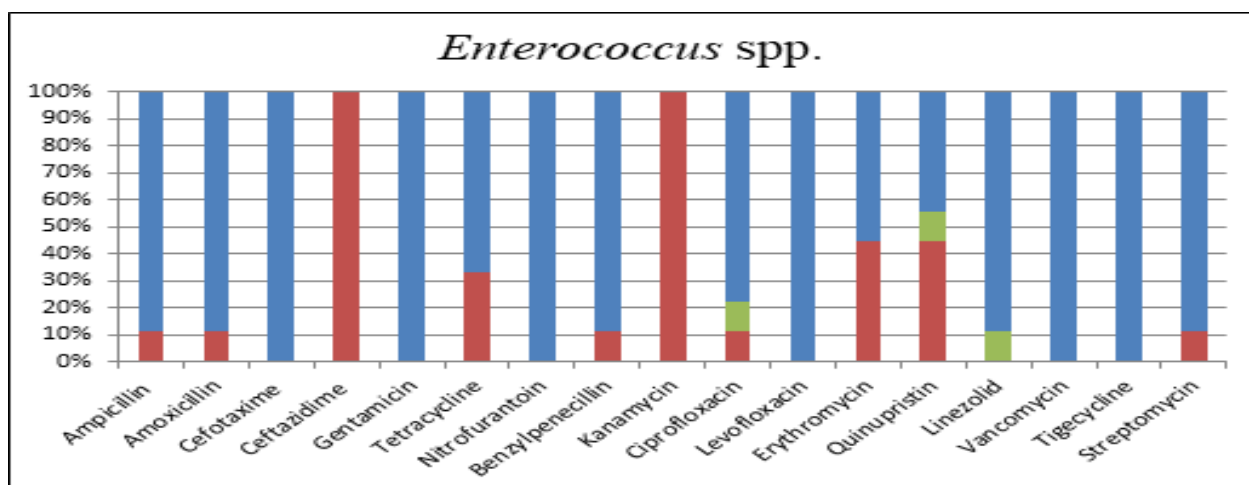


Figure 6. Results of Antibiotic Susceptible Testing of *Enterococcus* spp.

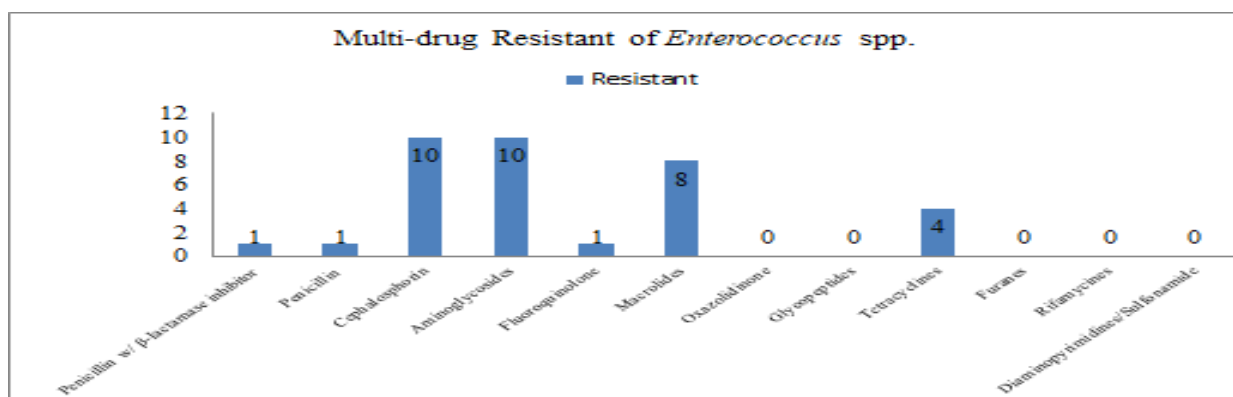


Figure 7. Multi-drug Resistance of *Enterococcus* spp.

Identified *Staphylococcus aureus* were resistant to antibiotics namely amoxicillin (100%), benzyl penicillin (100%), Cefotaxime (75%), Ceftazidime (75%), Oxacillin (80%), and Clindamycin (60%). Thus, it is susceptible to Gentamicin (100%), Tetracycline(90%), Nitrofurantoin (90%), Kanamycin (90%), Ciprofloxacin (90%), Levofloxacin (90%), Quinupristin(90%), Linizoid (90%), Rifampin (90%) shown in Figure 8.

Additionally, the identified *Staphylococcus aureus* isolates were identified as Multi-drug Resistant (Figure 9). Same number of isolates (6) was MDR to Penicillin and Penicillin with beta lactamase class of antibiotics. Followed by Cephalosporin class of antibiotics with Four (4) *S. aureus*. Lastly, with 3 *S.aureus* isolates was identified as MDR to Macrolides and Diaminopyrimidines/sulfamide.

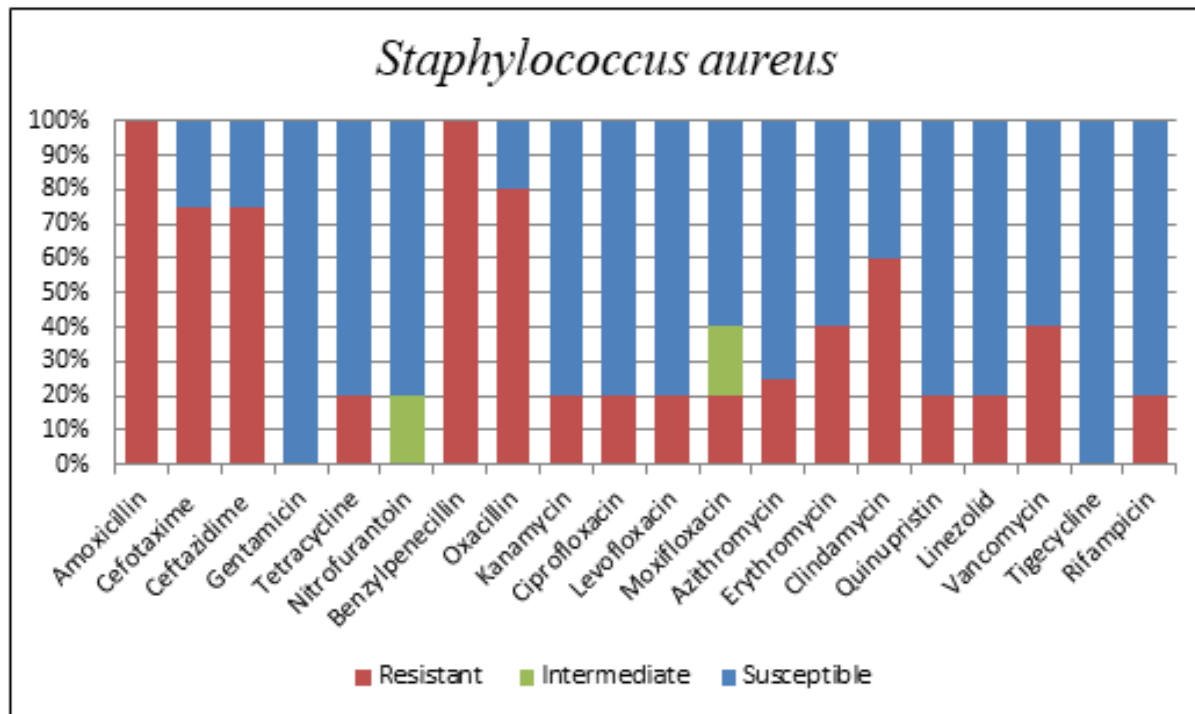


Figure 8. Results of Antibiotic Susceptible Testing of *Staphylococcus aureus*

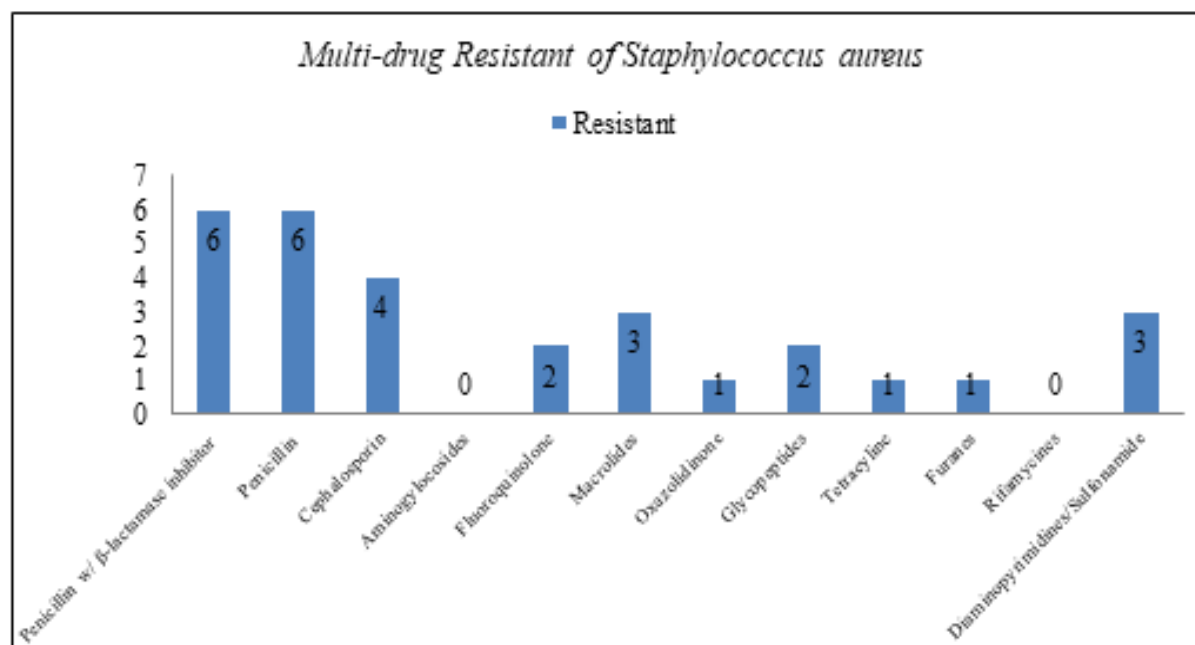


Figure 9. Multi-drug Resistant of *Staphylococcus aureus*

This study support the results of Walsh and Wenciewicz (2020) and McGuinness *et al.* (2017) that the resistance rate of *S. aureus* isolates to Penicillin is currently 90% or higher that concludes the inefficiency of this antibiotics to treat infections caused by this bacterial pathogen. *Staphylococcus aureus* demonstrated resistance to key first-line antibiotics such as penicillin, Amoxicillin and Oxacillin, suggesting potential MRSA presence (Pistella *et al.*, 2016).

Due to inexpensive and availability of the first-line antibiotics such as penicillin and Amoxicillin, the *S.aureus* became resistant. The resistance of *S.aureus* in the said antibiotics in Site 1 may be due to overuse and misuse of antibiotics as it is commonly consumed for self- medication

Furthermore, antimicrobial susceptibility testing (AST) revealed the emergence of Vancomycin-Resistant *Staphylococcus aureus* (VRSA) strains, which pose a serious threat due to their potential to cause severe health consequences when not effectively treated or contained. This is particularly alarming because vancomycin is widely regarded as a last-line therapy for treating methicillin-resistant *S. aureus* (MRSA) infections (Denissen *et al.*, 2022).

Results showed in Figure 10 that *Klebsiella pneumoniae* was highly resistant to Ampicillin, Cefalexin, Cefalotin, Doxycycline, Tetracycline, Cefovecin, Ceftaroline, Ceftiofur, and Sulfamethazole. However, *K.pneumoniae* was highly susceptible to Ceftazidime, Imepenim, Neomycin, Nalidixic Acid, Gentamycin, Marbofloxacin, Cefotaxime and Amoxicillin.

Similar to the study of Bereanu *et al.* (2024) resulted that *K. pneumoniae* was highly resistant or totally resistant to Penicillin class of antibiotics such as Ampicillin. Additionally, some of the class antibiotics cephalosporin, tetracycline, sulfonamide, phenicols, and furanes (Figure 11) were also resistant. In contrast to this study, *K.pneumoniae* was totally susceptible to type of antibiotic Imepenem-which is broad spectrum of beta lactams that often used as last resort of antibiotics for MDR gram-negative infection like *K.pneumoniae* (Girometti *et al.*, 2014; Martin & Bachman, 2018).

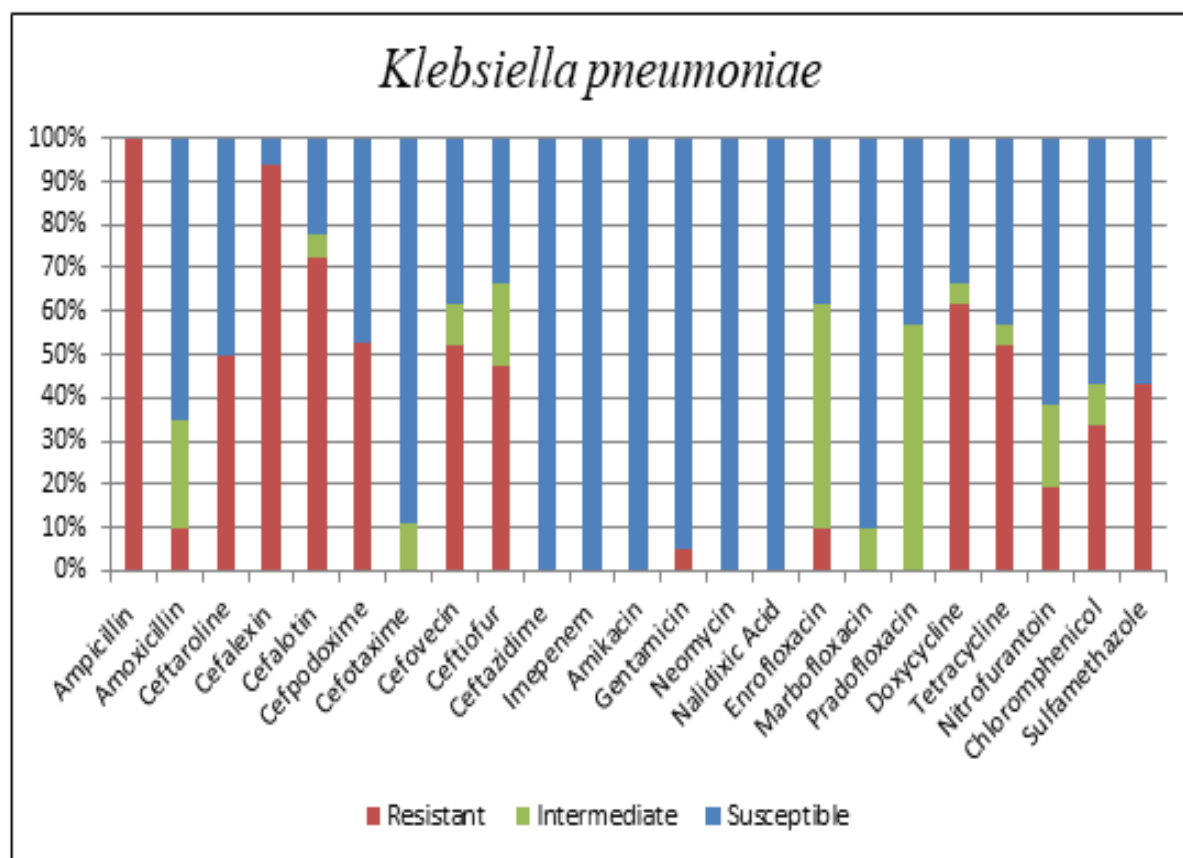


Figure 10. Results of Antibiotic Susceptible Testing of *Klebsiella pneumoniae*

Klebsiella pneumoniae isolates obtained in the two hospitals is identified as Multi-drug Resistance to antibiotics class of the following; Penicillin, Cephalosporin, Tetracycline, Diaminopyrimides/Sulfamide, Phenicols, Furanes and Penicillin with beta lactamase inhibitor (Figure 11).

Ten out of 21 *K. pneumoniae* isolates tested positive for ESBL. Furthermore, all twenty-one (21) *Klebsiella pneumoniae* isolates exhibited resistance to ampicillin and showed an extensive resistance profile, particularly to Penicillin/beta-lactams and some cephalosporin antibiotics which support the study of Pistella *et al.* (2016).

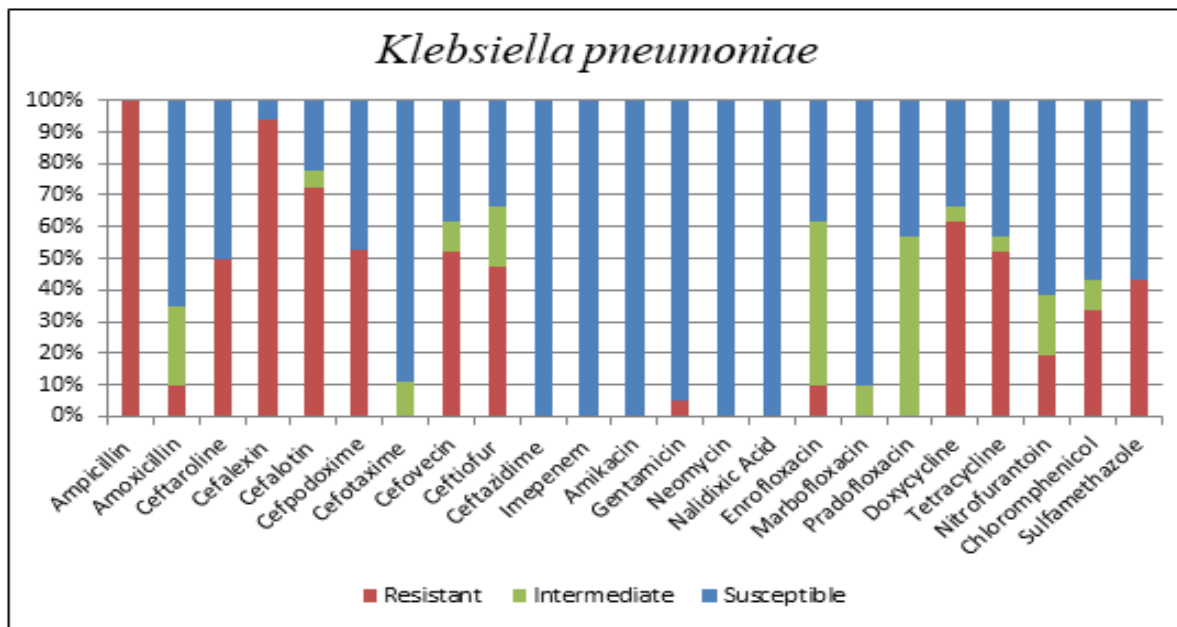


Figure 10. Results of Antibiotic Susceptible Testing of *Klebsiella pneumoniae*

Moreover, *K. pneumoniae* is fully susceptible to class of antibiotics such as Amikacin and Neomycin and highly susceptible to gentamicin, class Fluoroquinolones and Macrolides (Denissen et al., 2022).

The Multiple Antibiotic Resistance (MAR) Index was determined for both Site 1 and Site 2 to evaluate the potential health risks linked to the antibiotic-resistant isolates identified at each site.

The assessment of MAR index at Site 1 revealed values ranging from 0.04 to 0.67 across the three sampling stations in the hospital. A majority of the isolates, 25 out of 34 (73.53%), exhibited MAR indices of 0.2 or higher, indicating exposure to environments with frequent antibiotic use. In contrast, only 9 isolates (26.47%) had MAR values below 0.2, reflecting minimal antibiotic pressure.

At Site 2 (Visayas), the MAR index of the isolates ranged from 0.18 to 0.29 across the three sampling stations, with an average index value of 0.24. Of the four isolates collected, three exhibited MAR indices of ≥ 0.2 , indicating possible exposure to high-risk antibiotic environments, while one isolate had a MAR index of ≤ 0.2 .

The MAR index values at both sampling sites — 0.50 for Site 1 and 0.36 for Site 2— exceeded the threshold of ≤ 0.2 . Bacteria isolated from both hospitals showed resistance to multiple antibiotics, indicating possible exposure to high-risk environments with frequent antibiotic use or contamination (Kruperman, 1983). These findings highlight the urgent need for antimicrobial resistance monitoring and the implementation of infection control measures in both facilities.

CONCLUSIONS

In conclusion, a total of 38 bacterial isolates were identified as *Enterococcus* spp., *Staphylococcus aureus*, and *Klebsiella pneumoniae* in two sampling sites with three stations in each hospital. In Mindanao (Site1) 34 bacterial isolates was obtained. *Klebsiella pneumoniae* was dominant with 21 isolates. Followed by *Enterococcus* species with 10 isolates and less dominant was the *Staphylococcus aureus* with 6 isolates. While in the Visayas (Site 2) there were only 4 isolates was obtained that may indicate there effective infection control and less contamination of the environment.

The number of *K. pneumoniae* obtained in the Mindanao (Site 1) was very alarming because this species was primarily an opportunistic pathogen that attacks immune-compromised individuals such as children specially neonates. Additionally, *K. pneumoniae* strains become resistant to 95% of the antimicrobials of the pharmaceutical market.

Most of the isolates exhibited a wide range of antibiotics resistance patterns, with high resistance to first-line of antibiotics such as Ampicillin, Amoxicillin, Benzyl penicillin, Oxacillin, Cefalexin, Cefalotin, Ceftazidime, and Cefpodoxime. That concludes the difficulty of treatment on such antibiotic therapies.

Lastly, the assessment on MAR index showed in Mindanao (Site 1) and Visayas (Site 2) had 0.50 and 0.36, respectively. Additionally, the MAR index values were 0.30 (Site 1) and 0.09 (Site 2). This concludes that the isolates may originate from high-risk environment with frequent antibiotic use or contamination.

Author Contributions

Ms. Saudah Asum Mauna conducted the research and writing the final paper. While, all the other authors contributed during the sampling period, collection of the data, laboratory works and enhancement of this paper.

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