Antimicrobial susceptibility and resistance genes profiles in gram negative isolates from automated teller machines in Cape Coast, Ghana

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Abstract

The prevalence and type of microorganisms present on surfaces is an important factor to consider in infection outbreaks. The study identified bacterial isolates from Automated Teller Machines (ATMs) and screened the isolates against antibiotics of aminoglycosides, penicillins, tetracyclines, and phenicols classes for efficacy. The isolates were further tested for presence of genes that encodes for aminoglycosides, tetracyclines, glycopeptides, penicillins, and beta-lactamases. The bacterial isolates were identified as clinical isolates of Enterobacteriaceae and Pseudomonadaceae and frequently comprised Escherichia coli and Klebsiella spp. The isolates were more susceptible to gentamicin and kanamycin (95%) and more resistant to flucloxacillin (65%), cloxacillin (65%), and oxytetracycline (60%). Most of the isolates showed multiresistance to the antibiotics and were commonly associated with Providencia stuartii, Klebsiella pneumoniae, Serratia marcescens, and Pseudomonas alcaligenes. The blaTEM (90%) and strA (80%) encoding genes were the most abundant genes within the isolates. Klebsiella pneumoniae, K. aerogenes, Enterobacter cloacae, Serratia marcescens, S. rubidaea, Yersinia mollaretii, and Salmonella Paratyphi co-habored 33% of carbapenemases, ESBLs, blaTEM, vanA, and strA encoding genes. This is the first study to highlight the emergence of antibiotic-resistant bacteria from ATMs as potential reservoir for emergence and spread of multidrug resistance genes in bacterial pathogens in Cape Coast, Ghana.

Keywords: Gram negative bacteria, Antimicrobials, Multidrug resistance, Resistance genes, Beta-lactamases

1. Introduction

Most microbes are harmless to the human body and form part of the human microbiome (Gibbons and Gilbert, 2015). Some microbes are useful in agriculture, industry and food production (Thomashow et al., 2019; and...
However, relatively few bacteria are classified as pathogens and are known to cause infectious diseases. Bacteria are responsible for some globally relevant human infectious diseases such as tuberculosis (Mycobacterium tuberculosis), pneumonia (Klebsiella pneumoniae), and cholera (Vibrio cholerae) which are associated with significant rates of morbidity and mortality (World Health Organization, 2019).

The discovery of antibiotics saw a rapid decrease in infectious disease mortalities (Jayachandran, 2018). However, the emergence of antibiotic resistance in the late 20th century saw a stall in the decreasing mortality and morbidity rates that came with the discovery of antibiotics in the early 20th century (Frieri et al., 2017). The increasing emergence and spread of antibiotic resistance in human bacteria pathogens are significant growing public health concerns and are gradually becoming a threat to development, food production, and ultimately life expectancy (Frieri et al., 2017; and von Wintersdorff et al., 2016).

Antibiotic resistance in bacteria pathogens results in higher medical costs, prolonged hospital stay, and ultimately increased mortality (Dadgostar, 2019; and World Health Organization, 2020). Tuberculosis was responsible for about 1.5 million deaths in 2018 globally and requires over $10 bn for treatment and management annually (World Health Organization, 2019). The advent of antibiotic resistance in bacteria pathogens threatens the ability to treat common infectious diseases. Consequently, common infections such as pneumonia and gonorrhea are becoming harder and sometimes impossible to treat (Ventola, 2015). Some developed countries have systems that control the spread of antibiotic resistance. However, resource constraints and lack of awareness of the scope of the threat limits the control of the emergence and spread of antibiotic resistance in developing countries (Newman et al., 2011).

It may seem strange to focus on antibiotics resistance amidst the COVID-19, a viral pandemic currently. However, the role of secondary infections coupled with antibiotic resistance in patients with COVID-19 cannot be underestimated (Li et al., 2020). Bacteria played a major role in deaths in earlier pandemics such as the H1N1 and SARS (Morris et al., 2017). With the recent increase in the misuse of antibiotics and the development of antibiotic resistance, bacteria superinfections are inevitable and increase the fatality rate of the pandemic (Ventola, 2015; and World Health Organization, 2020). Besides, nearly all COVID-19 patients receive antibiotics (Zhou et al., 2020). This further threatens the global crisis of antibiotic resistance.

Microbes can be transmitted indirectly from one individual to another through contaminated vehicles of transmission such as water and fomites (Stephens et al., 2019). This suggests that the development of infectious diseases from indirect contacts can easily be avoided when the intervening agents of transmission are identified. The use of electronic technology such as handheld devices and electronic point-of-care systems has been established as a source of contamination (Saroja et al., 2013). Automated Teller Machines (ATMs) are currently an important component of the banking sector as they make transactions easier and faster. For example, the decongestion and practicing of social distancing in banking halls as a result of the COVID-19 pandemic have led to increased reliance on ATMs by customers. This situation presents an increase in the transmission of infectious pathogens since customers are required to make physical hand interactions with the ATMs. Knowledge on the environment as a source and dissemination route for antibiotic-resistant bacteria is fundamental to identify risk scenarios for human health especially in Ghana (Pal et al., 2016). However, the pathogen retaining capacity of ATMs is relatively unexplored. Therefore, there is a need to assess the extent to which ATMs harbor and serve as transmission routes for antibiotic-resistant bacteria.

2. Materials and methods

2.1. Study area

The study was focused on the Cape Coast Metropolis (5°06’N, 1°15’W). Cape Coast is the regional capital of the Central Region of Ghana with a population of over 165,000. Owing to the numerous educational institutions in the metropolis, the rich culture, and the history of the town, it sees an influx of individuals, both local and international, who visit the town for educational and tourist purposes. This causes a periodic rise and fall in the population size of the metropolis. The Cape Coast Metropolis has several financial institutions some of which have ATMs located in different areas in the metropolis.

2.2. Sample size, sampling procedure, and sample collection

Approximately, 20 functional ATMs belonging to nine banks are in the Cape Coast metropolis. Using a combination of functions (Random - RAND, INDEX, and RANK) in Microsoft Excel 2016, 10 ATMs representing 50% were randomly selected for the study. The 10 ATMs were assigned numbers 1 (ATM_01) to 10 (ATM_10).
The samples were collected by gently swabbing sterile cotton swabs across the keys on the interaction panel of each ATM. The surface areas of the interaction panels averaged 300 cm². Each of the 10 ATMs was sampled three times over six months, from October 2019 to March 2020.

2.3. Isolation and identification of bacteria species

The samples were cultured in 1 ml sterile nutrient broth medium and incubated at a temperature of 37 ± 2°C for 24 h. The cultures were serially diluted at ten-fold dilution to the 10⁶ dilution factor. One-millimeter (1 ml) aliquot from the 10⁶ dilution factor of each sample was cultured in nutrient agar using the pour plate technique. The number of bacterial colonies in each Petri plate after incubation was recorded with a colony counter (Stuart Sci., UK). The isolates were identified based on colony morphology (form, surface, margin, appearance, and size), cell characteristics, gram staining, and biochemical tests (Triple sugar iron test, motility test, catalase test, indole test, and citrate test). A distinct colony of each identified isolate was sub-cultured for antibiotic susceptibility test and detection of the presence of antibiotic and beta-lactamase genes.

2.4. Phenotypic detection of antibiotic resistance

The antimicrobial susceptibility profile of each bacterial isolate was tested on Mueller-Hinton (MH) agar plates following the Kirby Bauer disk diffusion technique. Twenty microliters (20 µl) aliquot of each isolate was cultured on MH plates. Filter paper discs (0.6 cm) containing antibiotics were placed onto the plates using sterile forceps. The plates were allowed to stand for 1 min and then incubated at 37 ± 2°C for 24 h. The antibiotics used in the study comprised ampicillin (10 µg), cloxacinil (10 µg), and fluofoxacinil (10 µg) (penicillins class), gentamicin (30 µg) and kanamycin (30 µg), (aminoglycosides class), oxytetracycline (30 µg) (tetracyclines class), and chloramphenicol (10 µg) (phenicol class). These antibiotics are commonly used for the treatment of bacterial infections (Giler-Molina et al., 2020; and Rangarajan and Venkataraman, 2020). The zones of inhibition were measured and interpreted according to the guidelines by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org).

2.5. Genomic DNA extraction

The genomic DNA extraction was performed based on a modified version of the method of González-Mendoza et al. (2010). An aliquot (4 ml) of each isolate was centrifuged at 14,000 rpm for 10 min. The supernatants were discarded and 300 µl of extraction buffer (3.0% SDS, 1.0 M NaCl, 0.5 mM EDTA, 0.1 M Tris-HCl, pH 8.0), and glass beads were added in the Eppendorf tubes and vortexed for 2 min. Chloroform-phenol (300 µl) was added to each tube and incubated at 65°C for 10 min. The mixtures were allowed to cool to room temperature (25 ± 2°C) and centrifuged at 14,000 rpm for 5 min. The supernatants were transferred into new tubes. An equal volume of ice-cold isopropanol was added to each tube and mixed gently by inversions. The tubes were then incubated at -20°C for 30 min and centrifuged at 14,000 rpm for 10 min. The supernatants were discarded and the pellets were washed two times with 70% ethanol. The genomic DNAs were eluted in 30 µl Tris EDTA (TE) buffer and resolved in 1% agarose gel at 80 V for 40 min.

2.6. Detection of genes encoding antibiotic resistance

Beta-lactamases (bla) is the main mechanism of antibiotic resistance in bacteria (Zeng and Lin, 2013). The presence of beta-lactamase genes bla_{TFX48} and bla_{VM} (carbapenemases), bla_{CTXM} and bla_{TEM} (extended-spectrum beta-lactamase) and bla_{VIM} (further beta-lactamase), and a penicillin (meA), aminoglycoside (strA), tetracycline (tet(A)), and glycopeptide (vanA) resistance gene in the isolates were detected using primers (Table S1). The polymerase chain reaction (PCR) consisted of 5 µl of Taq PCR Master Mix (BBI Life Sci. Corp., China), 1 µl of genomic DNA, 0.5 µl each of forward and reverse primer and 3 µl of molecular gradewater. For a negative control reaction mix, sterile distilled was used in place of genomic DNA. The amplification was performed in a thermocycler (BIO-RA D T100™) under the following conditions: Pre-denaturation at 94°C for 4 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 55–58°C for 30 s and extension at 72°C for 1 min, and final extension at 72°C for 10 min. The PCR products were resolved in 2% agarose gel, visualized under ultraviolet (UV) light, and scored for presence or absence of the resistance genes using a 100 bp DNA ladder.

2.7. Data analyses

The qualitative data from the study were presented using descriptive statistics. One-way analysis of variance was used to test for variations in mean bacterial loads at a 5% level of significance using Genstat v21 statistical
software (VSNi, UK). Significance in mean bacterial loads was separated using Duncan’s multiple range test at a 5% level of significance.

3. Results

3.1. Estimation of total bacterial loads

The ATMs had significant variations \( (p < 0.001) \) in total bacterial loads with an average load of \( 7.89 \times 10^6 \text{ CFU ml}^{-1} \) (Figure 1). ATM_04 recorded the highest bacterial load of \( 18.90 \times 10^6 \text{ CFU ml}^{-1} \). In contrast, the lowest load of \( 2.70 \times 10^6 \text{ CFU ml}^{-1} \) was obtained for bacterial isolates from ATM_02. High bacterial loads were also observed in ATM_03 \( (13.60 \times 10^6 \text{ CFU ml}^{-1}) \) and ATM_09 \( (12.95 \times 10^6 \text{ CFU ml}^{-1}) \). Similar bacterial loads of \( 4.15 \times 10^6 \text{ CFU ml}^{-1} \), \( 4.70 \times 10^6 \text{ CFU ml}^{-1} \), \( 4.80 \times 10^6 \text{ CFU ml}^{-1} \) and \( 4.85 \times 10^6 \text{ CFU ml}^{-1} \) were also recorded for isolates from ATMs_10, 08, 05 and 06, respectively. ATM_07 and ATM_01 also gave similar bacterial loads of \( 6.95 \times 10^6 \text{ CFU ml}^{-1} \) and \( 5.25 \times 10^6 \text{ CFU ml}^{-1} \), respectively. There were no significant differences \( (p > 0.05) \) in the loads of bacterial isolates from ATMs_01, 05, 06, 08, and 10. Similarly, a significant difference was not observed between the bacterial loads of ATM_03 and ATM_09.

![Figure 1: Total bacterial loads of isolates from the ATMs](image)

3.2. Identification of bacterial isolates

A total of 20 bacterial isolates corresponding to 14 distinct species were identified from the ATMs (Figure 2, Table 1). Interestingly, all the bacterial species were gram-negative and rod shaped and belong to the Enterobacteriaceae (93%) and Pseudomonadaceae (93%) families. Two pathogens, Klebsiella pneumoniae, and Enterobacter spp, belonging to the six bacterial pathogens (ESKAPE) commonly associated with antimicrobial resistance \( (\text{Ciofu and Tolker-Nielsen, 2019}) \), were identified from ATMs_01 and 06, and ATM_02, respectively. Escherichia coli was the most common isolate (20%) and was found in ATMs_01, 02, 07, and 08. Providencia alcalifaciens (10%) and Salmonella Paratyphi (10%) were common to ATM_02 and ATM_10, ATM_06 and ATM_09, respectively. The remaining nine species (45%) identified were distinct to an ATM. Two species of Serratia (S. rubidaea and S. marcescens) were from ATM_03 and ATM_04. Klebsiella aerogenes and Shigella sonnei were associated with ATM_03 and ATM_05, respectively. ATM_07 similarly showed presence of Yersinia mollaretii and Proteus mirabilis, respectively. The other species of Enterobacteriaceae that were identified were Moellerella wisconsensis (ATM_06) and Providencia stuartii (ATM_10). The isolates from ATM_08 also revealed presence of Pseudomonas alcaligenes which belongs to the Pseudomonadaceae family.
**Figure 2: Bacteria isolates from ATMs**

**Table 1: Resistance and susceptibility patterns of the bacterial isolates to antibiotics**

<table>
<thead>
<tr>
<th>ATM</th>
<th>Bacterial isolate</th>
<th>Amp</th>
<th>Chl</th>
<th>Clo</th>
<th>Flu</th>
<th>Oxytet</th>
<th>Gen</th>
<th>Kan</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM_01</td>
<td>Klebsiella pneumoniae</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td>ATM_01</td>
<td>Escherichia coli</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td>ATM_02</td>
<td>Escherichia coli</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>ATM_02</td>
<td>Enterobacter cloacae</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>ATM_03</td>
<td>Serratia rubidae</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td>ATM_03</td>
<td>Klebsiella aerogenes</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td>ATM_04</td>
<td>Serratia marcescens</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td>ATM_05</td>
<td>Shigella sonnei</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
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<tr>
<td>ATM_05</td>
<td>Moellerella wisconsinis</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
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<tr>
<td>ATM_06</td>
<td>Salmonella Paratyphi</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
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<tr>
<td>ATM_06</td>
<td>Klebsiella pneumoniae</td>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
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<tr>
<td>ATM_07</td>
<td>Yersinia mollaretii</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
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<tr>
<td>ATM_07</td>
<td>Proteus mirabilis</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
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<tr>
<td>ATM_07</td>
<td>Escherichia coli</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
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<td>S</td>
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<tr>
<td>ATM_08</td>
<td>Escherichia coli</td>
<td>S</td>
<td>S</td>
<td>R</td>
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<tr>
<td>ATM_08</td>
<td>Pseudomonas alcaligenes</td>
<td>R</td>
<td>S</td>
<td>R</td>
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<tr>
<td>ATM_09</td>
<td>Providencia alcalifaciens</td>
<td>S</td>
<td>S</td>
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Table 1 (cont.)

<table>
<thead>
<tr>
<th>ATM</th>
<th>Bacterial isolate</th>
<th>Amp</th>
<th>Chl</th>
<th>Clo</th>
<th>Flu</th>
<th>Oxytet</th>
<th>Gen</th>
<th>Kan</th>
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</thead>
<tbody>
<tr>
<td>ATM_09</td>
<td>Salmonella Paratyphi</td>
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<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
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<td>S</td>
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<tr>
<td>ATM_10</td>
<td>Providencia stuartii</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td>ATM_10</td>
<td>Providencia alcalifaciens</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>


3.3. Resistance and susceptibility patterns of bacterial isolates

Based on the responses to the antibiotics, the bacterial isolates were classified as either resistant or susceptible to an antibiotic according to the guideline of EUCAST (Table 1). The bacterial species were more susceptible (67%) than resistant (33%) to antibiotics. The resistance and susceptibility patterns were independent of the antibiotics' classes and species' type. The isolates were more resistant to cloxacillin and flucloxacillin (65%), followed by oxytetracycline (60%). Resistance to florfenicol and ampicillin was recorded for 20% and 10% of the isolates and was evident in 50 Klebsiella aerogenes, Klebsiella pneumoniae (ATM_06), Serratia marcescens, and P. stuartii, and Pseudomonas alcaligenes and P. stuartii, respectively. All the bacterial isolates were susceptible to gentamicin and kanamycin except Salmonella Paratyphi isolated from ATM_06. P. stuartii showed the most resistance (71%) to all the antibiotics. In contrast, Providencia alcalifaciens was susceptible to all the antibiotics. Thirty percent of the isolates (Moellerella wisconsensis, Shigella sonnei, Escherichia coli (ATM_01 and 07), Providencia alcalifaciens (ATM_09), and Salmonella Paratyphi (ATM_09)) also showed single resistances to oxytetracycline (83%) and flucloxacillin (17%). High resistances of 57% were recorded for Klebsiella pneumoniae from ATM_06, Pseudomonas alcaligenes, and Serratia marcescens and were commonly observed in cloxacillin, flucloxacillin, oxytetracycline, and chloramphenicol.

3.4. Detection of genes that encode antibiotic resistance

The presence or absence of beta-lactamase genes bla<sub>OXA-48</sub> and bla<sub>VIM</sub> (carbapenemases), bla<sub>TEM</sub>, and bla<sub>CTX-M</sub> (EBSLs), mec<sub>A</sub>, and mec<sub>A</sub>, strA, tet(A), and vanA genes in the isolates were determined using primers specific to the genes (Table 2). The bla<sub>TEM</sub> and strA genes were the most predominant and were found in 90% and 80%
of the isolates. However, none of the isolates showed the presence of bla\textsubscript{CTX-M}, tet\textsubscript{(A)}, and mec\textsubscript{A} genes. The presence of bla\textsubscript{ampC} was high (35%) within the isolates. The genes bla\textsubscript{OXA-48}, bla\textsubscript{VIM}, and van\textsubscript{A} were present in Serratia marcescens and Enterobacter cloacae (10%), Serratia rubidaea and Klebsiella pneumoniae from ATM\_01 (10%), and Yersinia mollareti and Salmonella Paratyphi from ATM\_06, respectively. The Klebsiella, Enterobacter, Serratia, Salmonella, and Yersinia species had the highest number of genes (33%) among the isolates. In contrast, the Providencia species (P. stuartii and P. alcalifaciens) had the least number of genes. About 45% of the isolates, predominantly Escherichia coli, were positive for 22% of the genes. The isolates (45%) similarly exhibited co-occurrence of the resistance genes.

4. Discussion

The physical surfaces in built environment play a significant role in the spread of infectious diseases (Edemekong et al., 2020). In this study, we investigated the antibiotic resistance profile of bacteria species isolated from the interaction panels of ATMs. The highest bacterial load of 18.90 × 10\textsuperscript{6} CFU ml\textsuperscript{-1} from ATM\_04 may be due to a high number of people patronizing this ATM. The ATM is situated at the regional head office of one of the largest banks in Ghana. Moreover, the area is closer to one of the most popular tourist sites in the region and is found within the commercial zone of the Cape Coast metropolis. Even though the ATM\_02 is located on a commercial street, it had the lowest bacterial load of 2.70 × 10\textsuperscript{6} CFU ml\textsuperscript{-1}. This observation may be due to low patronage by customers. The finding is similar to the study by Saroja et al. (2013) who reported the presence of bacteria contaminations in ATMs in Chennai, India with a relatively low range of 40.0 CFU ml\textsuperscript{-1} to 9.7 × 10\textsuperscript{6} CFU ml\textsuperscript{-1}.

The ATM\_03 and ATM\_09 are also located in densely populated zones of the metropolis and recorded similar and relatively higher bacterial loads. Similar amounts of bacterial loads were found in 50% of the ATMs (ATMs\_01, 05, 06, 08, and 10), indicating that these ATMs, as well as ATMs\_03 and 09, pose similar levels of infection, respectively, when customers use them. The findings may suggest that there exists a relationship between the level of bacterial contamination on an ATM and the frequency of usage of the ATM. Earlier studies have reported that the diversity and number of customers who use ATMs influence the microbial loads on ATMs (Nworie et al., 2012). The low level of awareness of the capacity of contact surfaces to retain and serve as a source of medium for transfer of microbes coupled with a rudimentary hand hygiene practice in Ghana could be associated with the high level of bacterial contamination observed on the ATMs (Roberts et al., 2013).

<table>
<thead>
<tr>
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<th>Bacterial isolate</th>
<th>bla\textsubscript{ampC}</th>
<th>bla\textsubscript{CTX-M}</th>
<th>bla\textsubscript{OXA-48}</th>
<th>bla\textsubscript{VIM}</th>
<th>bla\textsubscript{TEM}</th>
<th>mec\textsubscript{A}</th>
<th>strA</th>
<th>tet\textsubscript{(A)}</th>
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<td>ATM_09</td>
<td>Salmonella Paratyphi</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ATM_10</td>
<td>Providencia stuartii</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ATM_10</td>
<td>Providencia alcalifaciens</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

Percentage: 35% / 65% / 100% / 10% / 90% / 100% / 90% / 20% / 100% / 10% / 90%

Note: – : Absence of gene; and + : Presence of gene.
Escherichia coli was the most abundant Enterobacteriaceae followed by Klebsiella species (K. pneumoniae and K. aerogenes), Providencia species (P. alcalifaciens and P. stuartii), Serratia species (S. marcescens and S. rubidaea), and Salmonella Paratyphi. The other species of the group were Shigella sonnei, Enterobacter cloacae, Moorella visconsensis, Yersinia mollaretii, and Proteus mirabilis. Pseudomonas alcaligenes was the only species of Pseudomonadaceae found, suggesting a low prevalence of the group in isolates from ATMs. Previous studies have reported variations in the number and types of bacteria species isolated from ATMs (Odeyemi et al., 2018; and Okoro et al., 2018). Mahmoudi et al. (2017) reported an average of seven isolates in 96 ATMs studied in western Iran. A study conducted on four ATMs within the Calabar metropolis of Nigeria identified 79 bacteria isolates (Agú et al., 2018).

Even though ATM_04 had the highest bacterial load, only one species (S. marcescens) was found from the ATM compared with the remaining ATMs which had low to high bacterial loads and at least two bacterial species each. This indicates that microbial populations compete and contra-inhibit their exponential growth and survival rates. The study of Allen and Wadaw (2018) indicated that bacteria interactions cause competition for space between lineages. There is an increasing prevalence of all the bacterial species found from this study as pathogens and frequently associated with healthcare outbreaks (such as urinary tract infections, lung diseases, acute diarrhea, and neonatal sepsis) and increased morbidity and mortality (Ahmad et al., 2020; and Sands et al., 2021).

To ascertain the antibiotic resistance pattern of the bacterial isolates, a combined approach of phenotypic and genotypic assaying was employed. The isolates responded differently to antibiotics of the aminoglycosides (gentamicin and kanamycin), tetracycline (oxytetracycline), phenicols (chloramphenicol), and penicillins (flucloxacillin, ampicillin, and cloxacillin) classes. The high resistance of the bacterial isolates to oxytetracycline, flucloxacillin, and cloxacillin suggests that flucloxacillin, cloxacillin, and oxytetracycline may be less effective at controlling these bacterial isolates. The presence of low resistances of the bacterial isolates to chloramphenicol, gentamicin, and kanamycin has been reported (Simsek, 2019). The resistance of Salmonella Paratyphi from ATM_06 to both kanamycin and gentamicin indicates cross-resistance between these aminoglycoside antibiotics. Cross-resistance among aminoglycoside antibiotics including gentamicin, kanamycin, and streptomycin has similarly been reported by Zhang et al. (2015).

In contrast, gentamicin and kanamycin were the most potent against the isolates with 95% of isolates being susceptible. This observation contradicts the previous reports that indicated aminoglycoside antibiotics such as kanamycin and gentamicin have become less effective against bacteria (Krause et al., 2016). Cross-resistances to the antibiotics were also observed for most of the bacterial isolates. The high resistance of P. stuartii to the antibiotics indicates an increasing antibiotic resistance in P. stuartii. Warda et al. (2018) has similarly reported multidrug resistance (MDR) in P. stuartii. This corroborates the current increasing prevalence of P. stuartii infections (Liu et al., 2020). The susceptibility of all the Salmonella Paratyphi isolates to chloramphenicol has similarly been observed by Maharjan et al. (2021).

The level of diversity in Antibiotic Resistance Genes (ARGs), particularly in human pathogens, does not depend on gene abundance and the number of samples (Bengtsson-Palme, 2018). Moreover, a small set of ARGs could provide an insight into the total diversity of resistance genes (Bengtsson-Palme, 2018). The most common genes detected in the isolates were the blaTEM, and strA genes. The study by Sheikh et al. (2012) concluded that blaTEM genes are highly associated with strA genes. These genes are responsible for transferable ampicillin and penicillin (blaTEM), and streptomycin (strA) resistances (Alcock et al., 2020). The observed recurrence of the blaTEM and strA genes in the bacterial isolates aligns with previous study in a tertiary care center in India (Grover et al., 2013). Bacteria species that harbored blaPRE genes (bla class C) were high compared with the carbapenemase genes blaOXA-48 (bla class D) and blaVIM (bla class B), and vanA genes which were present in few species. These genes have catalytic efficiency against cephalothin, cefazolin, and most penicillins cefoxitin (blaPRE), carbapenems comprising imipenem, meropenem, doripenem, and ertapenem (blaOXA-48 and blaVIM), and vancomycin (vanA), (A redondo-Alonso et al., 2021; and Sakkas et al., 2019).

Even though 60% of the bacterial isolates were resistant to tetracycline, none of the isolates had the tet(A) gene, as well as the blaCTX-M and mecA genes, present. Besides, blaTEM and blaCTX-M belong to the same class (A) of beta-lactamases. This suggests that antibiotics belonging to the same class may exhibit different actions against bacteria species (Wang and Lipsitch, 2006). The tet(A), mecA, and blaCTX-M genes code for the ability of bacteria to hydrolyze tetracycline, methicillin, and beta-lactam antibiotics such as cefotaxime, ceftazidime, and aztreonam, respectively (Liu et al., 2020). Escherichia coli was the most common isolate and
harbored only bla<sub>TEM</sub> and strA genes. This suggests the presence of an association between bla<sub>TEM</sub> and strA genes in the pathogen and confirms the findings of Sheikh et al. (2012) who concluded that bla<sub>TEM</sub> genes are highly linked to strA genes in Escherichia coli. Enterobacter cloacae exhibit bla<sub>OXA-48</sub> carbapenemase encoding genes (Solgi et al., 2020).

Despite the MDR of P. stuartii, the pathogen had the presence of only the ESBL bla<sub>TEM</sub> gene. P. stuartii showed the presence of only bla<sub>OXA-48</sub> and bla<sub>NDM</sub> genes when genotyped for four carbapenemase genes including bla<sub>TEM</sub> (Warda et al., 2018). The coexistence of the carbapenemase genes bla<sub>TEM</sub> and bla<sub>VIM</sub>, ESBL bla<sub>TEM</sub> gene, and bla<sub>TEM</sub> and vanA genes in all the isolates, except P. alcalifaciens from ATM_10 and P. stuartii, is threatening to public health. The Klebsiella pneumoniae isolates commonly exhibited bla<sub>TEM</sub> and strA and either bla<sub>VIR</sub> or bla<sub>TEM</sub>. Klebsiella pneumoniae isolates similarly tested positive for bla<sub>TEM</sub> and bla<sub>OXA-48</sub> but negative for bla<sub>VIR</sub> genes (Ferreira et al., 2019; and Solgi et al., 2020). The absence of bla<sub>OXA-48</sub> in K. pneumoniae as observed in the study has been documented (Ferreira et al., 2019). This suggests that bla<sub>TEM</sub> may be a common ESBL-encoding gene in K. pneumoniae. Proteus mirabilis showed bla<sub>TEM</sub> and strA genes and phenotypic resistance to flucloxacillin and cloxacillin. Current reports have highlighted the emergence and spread of MDR and antimicrobial resistance genes including beta-lactamases in P. mirabilis (Girlich et al., 2020). The presence of the bla<sub>OXA-48</sub> gene in Serratia marcescens concords with the outbreak of OXA-48-producing S. marcescens reported in Iran (Solgi et al., 2020). Shigella sonnei similarly tested positive for bla<sub>TEM</sub> and strA genes and negative for bla<sub>VIR</sub> genes in an earlier study in China (Wang et al., 2019).

5. Conclusion

Bacterial isolates from ATMs in the Cape Coast metropolis comprised Enterobacteriaceae and Pseudomonadaceae species that belonged to 11 genera. Most of the isolates exhibited multiple antibiotic resistance. Gentamicin and kanamycin were the most effective antibiotics against the bacterial isolates. The isolates revealed the presence of beta-lactamases and mecA genes and demonstrated a high potential for the acquisition of bla<sub>TEM</sub> and strA genes. This is the first study on antibiotic susceptibility and resistance gene pool of bacterial species from ATMs in the Cape Coast metropolis and further recommend proper cleaning of ATMs in the metropolis as well as hand sanitization by customers.

Conflicts of Interest

The authors declare no conflict of interest.

References


