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Characterization of Beta-Lactam Resistance in Clinical Pseudomonas aeruginosa and Escherichia coli Isolates from Trinidad and Tobago

Reinand Thompson[1]

reinand.thompson@sta.uwi.edu

The University of the West Indies, Trinidad and Tobago

Samantha Alexis[1]

The University of the West Indies, Trinidad and Tobago

Aleisha Ali[1]

The University of the West Indies, Trinidad and Tobago

Navin Ramdass

The University of the West Indies, Trinidad and Tobago

Zara Aziz

The University of the West Indies, Trinidad and Tobago

Kayren Sumai

The University of the West Indies, Trinidad and Tobago

Chandrashekhar Unakal[1]

The University of the West Indies, Trinidad and Tobago

Eberechi Patrick Akpaka[1]

The University of the West Indies, Trinidad and Tobago

Whiteney Arneud

University of the West Indies

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Abstract

Background

Beta-lactam resistance in clinically important Gram-negative bacteria is a major public health concern. Local surveillance integrating phenotypic antimicrobial susceptibility testing with molecular detection of resistance determinants is needed to clarify regional antimicrobial resistance patterns. This study investigated beta-lactam resistance in clinical *isolates of Escherichia coli and Pseudomonas aeruginosa* from Trinidad and Tobago.

Methods

A total of 154 *E. coli* and 132 *P. aeruginosa* isolates collected from two tertiary hospital systems in Trinidad and Tobago between 2021 and 2024 were phenotypically analysed by disc diffusion antimicrobial susceptibility testing. Resistant isolates were selected by stratified sampling for polymerase chain reaction screening of the beta-lactamase-associated gene families TEM, SHV, CTX, KPC, OXA, and IMP. Chi-square testing, odds ratios and 95% confidence intervals were used to compare resistance phenotypes and gene detection frequencies between species.

Results

Among E. coli isolates, 33.8% (52/154) were resistant to second- and/or third-generation cephalosporins, and 9.21% (14/154) were resistant to carbapenems. Among *P. aeruginosa* isolates, 23.5% (31/132) were resistant to second- and/or third-generation cephalosporins, and 23.5% (31/132) were resistant to carbapenems. Carbapenem resistance was significantly more frequent in *P. aeruginosa* than in *E. coli* ($\chi^2 = 11.11$, $p = 0.0009$; OR 3.07, 95% CI: 1.55–6.06). In the PCR-screened subset, CTX was detected in 52.0% (13/25) of *E. coli* isolates, and IMP was not detected in *any E. coli isolates*. Among *P. aeruginosa* isolates, SHV, CTX and OXA were each detected in 16.0% (4/25) isolates, while KPC and IMP were each detected in 12.0% (3/25) isolates.

Conclusions

Clinically relevant beta-lactam resistance was observed in both organisms, with a higher frequency of carbapenem resistance in *P. aeruginosa*. The differing gene detection profiles, including IMP detection only among *P. aeruginosa* isolates, suggest heterogeneous resistance mechanisms and support the need for continued local surveillance using broader molecular and sequencing-based approaches.

Background

Antibiotics, together with public health, infection control and sanitation measures, remain one of the most important defences against bacterial infection. However, the widespread and inappropriate use of antimicrobial agents has substantially contributed to the emergence and spread of antimicrobial resistance (AMR), particularly in settings where infectious disease burdens remain high [1]. AMR is associated with substantial morbidity, mortality, and rising healthcare costs, and projections suggest that this burden may increase further in the coming decades [2].

Among the antimicrobial classes affected by resistance, beta-lactam antibiotics are of particular importance due to their widespread clinical use. These agents include penicillins, cephalosporins, carbapenems, monobactams and related compounds, all of which share the beta-lactam ring as a core structural feature. Their antibacterial activity is mediated primarily through binding to penicillin-binding proteins, thereby inhibiting bacterial cell wall synthesis [3].

Bacteria may develop resistance to beta-lactam antibiotics through several mechanisms, including decreased outer membrane permeability, overexpression of efflux pumps, alteration of penicillin-binding proteins and production of beta-lactamase enzymes [4]. Among these mechanisms, beta-lactamase production is one of the most clinically significant. These enzymes hydrolyse the beta-lactam ring and thereby inactivate the antibiotic [5]. Beta-lactamases vary widely in substrate profile, amino acid sequence and molecular structure, and include extended-spectrum beta-lactamases, carbapenemases and other clinically important beta-lactamase families.

Two Gram-negative organisms of major clinical relevance in this context are *Escherichia coli* and *Pseudomonas aeruginosa*. *E. coli* is a common cause of urinary tract infections, bloodstream infections, intra-abdominal infections and healthcare-associated infections, while *P. aeruginosa* is an opportunistic pathogen associated with severe hospital-acquired infections, particularly in immunocompromised patients and in those with prolonged hospital stays, invasive devices or prior antibiotic exposure [6–7]. Both organisms have demonstrated a marked ability to acquire or develop resistance to multiple antimicrobial classes, including beta-lactams [8].

The molecular basis of beta-lactam resistance in these organisms is diverse. In *E. coli*, resistance is frequently associated with acquired beta-lactamase genes such as TEM, SHV and CTX-M, as well as carbapenemases in some settings [3, 9]. Carbapenemases are particularly important because they can compromise carbapenem therapy; clinically relevant groups include class A carbapenemases such as KPC, class D OXA-type enzymes and class B metallo-beta-lactamases such as IMP, VIM and NDM [10]. In *P. aeruginosa*, resistance may be mediated by acquired beta-lactamases, including metallo-beta-lactamases, as well as by strong intrinsic and adaptive mechanisms, such as inducible chromosomal beta-lactamase activity, porin loss, and multidrug efflux systems [7, 10–11]. Phenotypic resistance patterns may therefore not be fully explained by a limited panel [6, 8].

Although AMR is recognised as a global threat, local surveillance remains essential because resistance patterns vary by geographic region, institution, circulating strain type and antimicrobial use practices [8]. In Trinidad and Tobago and the wider Caribbean, published molecular AMR data remain limited but

include reports of KPC-producing *P. aeruginosa*, ESBL-associated genes in Enterobacterales and complex resistome features in *Klebsiella pneumoniae* [12–14]. There remains limited published data directly comparing phenotypic resistance patterns with the detection of selected beta-lactamase-associated genes in *E. coli* and *P. aeruginosa* from Trinidad and Tobago. This study aimed to evaluate the phenotypic and molecular characteristics of beta-lactam resistance in clinical isolates of *E. coli* and *P. aeruginosa* collected in Trinidad and Tobago. Specifically, the study aimed to determine the frequency of cephalosporin and carbapenem resistance and to screen resistant isolates for selected beta-lactamase-associated gene families, namely TEM, SHV, CTX, KPC, OXA and IMP.

Methods

Study design, setting and isolate selection

This was a laboratory-based cross-sectional observational study of clinical isolates *of E. coli* and *P. aeruginosa* obtained from two tertiary hospital systems in Trinidad and Tobago. A total of 154 *E. coli* isolates and 132 *P. aeruginosa* isolates were collected over a four-year period from 2021 to 2024 using a random sampling approach. Specimen types included urine, cerebrospinal fluid, wound swabs and blood. Initial bacterial identification was performed using conventional biochemical methods as described by Manafi [15], and species identity was subsequently confirmed prior to inclusion.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disc diffusion method as described by Jorgensen and Turnidge [16]. Each isolate was subcultured and incubated for 24 hours at 37°C. Bacterial colonies from overnight culture were suspended in sterile saline and adjusted to a turbidity equivalent to a 0.5 McFarland standard. The standardised inoculum was then inoculated onto Mueller-Hinton agar plates. Oxoid antimicrobial susceptibility test (AST) discs from different antibiotic classes (penicillins, second- and third-generation cephalosporins, and carbapenems) were applied to the inoculated M-H medium plates. The cultures were then allowed to grow, and the AST breakpoints and MICs were determined according to CLSI standards.

For subsequent molecular analysis, selected resistant isolates were screened for beta-lactamase-associated genes. Isolates demonstrating resistance to second- and/or third-generation cephalosporins and/or carbapenems were evaluated for TEM, SHV, CTX, OXA, KPC and IMP.

Molecular detection of selected beta-lactamase-associated genes

To investigate selected molecular determinants of resistance, 25 resistant *E. coli* isolates and 25 resistant *P. aeruginosa* isolates were selected for polymerase chain reaction (PCR) analysis using a stratified sampling approach. These were the same resistant isolate subsets used for the other beta-

lactamase-associated genes in the study. Isolates were grouped according to collection period (2021, 2022, 2023 and 2024) and specimen type (blood, wound swab, urine and cerebrospinal fluid), after which samples were randomly selected within each category. Molecular testing was performed on a subset due to laboratory resource constraints; the stratified approach was intended to preserve temporal and specimen-type representation within the subset tested.

DNA extraction

Genomic DNA was extracted using a modified heat lysis/boiling method based on the protocol described by Chalini et al. [17]. Briefly, three loopfuls of overnight pure culture were suspended in 200 µL of Tris-acetate-EDTA buffer in sterile microcentrifuge tubes. The suspension was vortexed for 5 seconds and incubated at 95°C for 20 minutes in a preheated thermomixer. The lysate was centrifuged at 12,000 rpm for 3 minutes. Following centrifugation, 150 µL of clear supernatant was transferred to a fresh labelled microcentrifuge tube and used as the DNA template for PCR.

PCR amplification

PCR amplification was performed using a Biometra T3 Prime thermal cycler. Reactions were prepared using the Promega Hot Start DNA Polymerase kit (Cat. No. M5001). Each 50 µL reaction mixture contained 10 µL of reaction buffer, 5 µL of $MgCl_2$, 1 µL each of forward and reverse primers, 2 µL of DNA template and 0.25 µL of Hot Start Taq DNA polymerase. Nuclease-free water was added to a final volume of 50 µL.

Primer sequences and PCR conditions for TEM, SHV, and CTX were based on published PCR assays [18]; those for KPC and IMP on Zowawi et al. [19]; and those for OXA on Rajivgandhi et al. [20]. Primer sequences, expected amplicon sizes and PCR cycling conditions are summarised in Table 1.

Gel electrophoresis and quality control

PCR products were analysed by electrophoresis on 1% agarose gels stained with GelRed. A DNA ladder was included in each run, and band sizes were estimated by comparison with the ladder and the published reference amplicon sizes for each target gene. *Klebsiella pneumoniae* ATCC 700603 was used as a positive control for SHV- and CTX-type genes, while *E. coli* ATCC 35218 was used as a positive control for TEM.

Statistical analysis

Phenotypic resistance frequencies and gene detection frequencies were summarised as counts and percentages. Chi-square testing was used to compare resistance phenotypes and PCR-detected gene frequencies between species. For gene-detection comparisons with small cell counts, the chi-square statistic was calculated with Yates continuity correction to maintain consistency across the molecular

analysis table. Odds ratios and 95% confidence intervals were calculated for comparisons of phenotypic resistance. A p-value < 0.05 was considered statistically significant.

Table 1
Primers and PCR conditions for selected beta-lactamase-associated genes

Gene	Primer sequence	PCR conditions	Expected amplicon size (bp)	Reference
OXA	Forward: 5'-ACACAATACATATCAACTTCGC-3' Reverse: 5'-AGTGTGTTTAGAATGGTGATC-3'	Initial denaturation at 95°C for 2 min; 30 cycles of 94°C for 30 s, 62°C for 90 s, and 72°C for 60 s; final extension at 72°C for 10 min.	813	Rajivgandhi et al. [20]
TEM	Forward: 5'-GTATCCGCTCATGAGACAATA-3' Reverse: 5'-TCTAAAGTATATATGAGTAAAC-3'	Initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 60 s, 55°C for 60 s and 72°C for 60 s; final extension at 72°C for 10 min.	966	Monstein et al. [18]
SHV	Forward: 5'-GCCGGGTTATTCTTATTTGTCGC-3' Reverse: 5'-ATGCCGCCGCCAGTCA-3'	Initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 68°C for 30 s and 72°C for 60 s; final extension at 72°C for 7 min.	1007	Monstein et al. [18]
CTX	Forward: 5'-SCSATGTGCAGYACCAGTAA-3' Reverse: 5'-CCGCRATATCRTTGGTGGTG-3'	Initial denaturation at 94°C for 5 min; 30 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 60 s; final extension at 72°C for 7 min.	543	Monstein et al. [18]
KPC	Forward: 5'-ATCTGACAACAGGCATGACG-3' Reverse: 5'-GACGGCCAACACAATAGGTG-3'	Initial denaturation at 95°C for 2 min; 35 cycles of 94°C for 30 s, 55°C for 45 s and 72°C for 1 min; final extension at 72°C for 5 min.	452	Zowawi et al. [19]
IMP	Forward: 5'-CTACCGCAGCAGAGTCTTTGC-3' Reverse: 5'-GAACAACCAGTTTTGCCTTACC-3'	Initial denaturation at 95°C for 2 min; 35 cycles of 95°C for 30 s, 58°C for 45 s and 70°C for 60 s; final extension at 70°C for 5 min.	591	Zowawi et al. [19]

Table legend: Primer sequences, cycling conditions and expected amplicon sizes are shown for each target included in the PCR panel.

Results

Phenotypic antimicrobial resistance by species

A total of 286 clinical isolates were included in the phenotypic antimicrobial susceptibility analysis. Of these, 53.8% (154/286) were *E. coli* and 46.2% (132/286) were *P. aeruginosa*.

Among *E. coli* isolates, 33.8% (52/154) demonstrated resistance to second- and/or third-generation cephalosporins, whereas 9.2% (14/154) were resistant to carbapenems. Among *P. aeruginosa* isolates, 23.5% (31/132) were resistant to second- and/or third-generation cephalosporins, and 23.5% (31/132) were resistant to carbapenems.

Comparison of cephalosporin resistance between species showed no statistically significant difference ($\chi^2 = 3.65$, p = 0.054; odds ratio [OR] 0.60, 95% confidence interval [CI]: 0.36–1.02). In contrast, carbapenem resistance differed significantly between *E. coli* and *P. aeruginosa* ($\chi^2 = 11.11$, p = 0.0009; OR 3.07, 95% CI: 1.55–6.06), with carbapenem resistance being more frequent in *P. aeruginosa*.

Table 2
Comparison of cephalosporin and carbapenem resistance

Antibiotic class	*E. coli* (n = 154)	*P. aeruginosa* (n = 132)	χ^2	p-value	OR	95% CI
Cephalosporins	52 (33.8%)	31 (23.5%)	3.65	0.054	0.60	0.36–1.02
Carbapenems	14 (9.21%)	31 (23.5%)	11.11	0.0009	3.07	1.55–6.06

Table legend: Statistical comparisons were performed between species for each antibiotic class.

Molecular detection of selected beta-lactamase-associated genes

To investigate selected molecular determinants of resistance, 25 resistant *E. coli* isolates and 25 resistant *P. aeruginosa* isolates were screened by PCR for the presence of TEM, SHV, CTX, KPC, OXA, and IMP.

Among the 25 *P. aeruginosa* isolates analysed, SHV, CTX and OXA were each detected in 4 isolates, while KPC and IMP were each detected in 3 isolates. Among the 25 *E. coli* isolates analysed, TEM and SHV were each detected in 4 isolates, CTX in 13 isolates and OXA in 7 isolates; KPC and IMP were not detected in *E. coli*, as depicted in Table 3.

The molecular analysis (Figs. 2 and 3) revealed distinct gene detection profiles between the two species. In *P. aeruginosa*, SHV, CTX, OXA, KPC and IMP were detected. In *E. coli*, CTX was the most frequently

detected target, together with TEM, SHV and OXA, along with TEM, SHV, and OXA, whereas KPC and IMP were not detected in the isolates screened.

CTX detection was significantly more frequent in *E. coli* than in *P. aeruginosa* ($\chi^2 = 5.704$, $p = 0.0169$). No statistically significant association was observed between species and detection of TEM, SHV, OXA, KPC or IMP, although differences in raw frequencies were noted.

Table 3
Selected beta-lactamase-associated gene detection by species

Gene	*E. coli* positive (n = 25)	*P. aeruginosa* positive (n = 25)	χ^2	p-value
TEM	4 (16%)	0 (0%)	2.446	0.1179
SHV	4 (16%)	4 (16%)	0.000	1.0000
CTX	13 (52%)	4 (16%)	5.704	0.0169
OXA	7 (28%)	4 (16%)	0.466	0.4947
KPC	0 (0%)	3 (12%)	1.418	0.2337
IMP	0 (0%)	3 (12%)	1.418	0.2337

Table legend: Percentages were calculated using the number of PCR-screened isolates of each species as the denominator. Statistical comparisons between species for each gene were performed using chi-square testing with Yates' continuity correction.

Discussion

This study provides a combined phenotypic and molecular assessment of beta-lactam resistance in clinical *P. aeruginosa* and *E. coli* isolates from Trinidad and Tobago. The findings demonstrate clinically relevant levels of resistance to second- and third-generation cephalosporins in both species, together with a significantly higher frequency of carbapenem resistance in *P. aeruginosa* than in *E. coli*. Molecular analysis of selected resistant isolates identified several beta-lactamase-associated gene families, including SHV, CTX, OXA, KPC and IMP in *P. aeruginosa*, and TEM, SHV, CTX and OXA in *E. coli* [10, 12–14, 21].

Phenotypically, 23.5% of *P. aeruginosa* isolates demonstrated resistance to second- and/or third-generation cephalosporins, while 23.5% were resistant to carbapenems. In contrast, 33.8% of *E. coli* isolates were resistant to second- and/or third-generation cephalosporins, whereas 9.2% were carbapenem resistant. These findings suggest that while cephalosporin resistance is substantial in both organisms, carbapenem resistance is a particular concern in *P. aeruginosa*. This is biologically plausible because *P. aeruginosa* possesses multiple intrinsic and adaptive resistance mechanisms, including reduced outer membrane permeability, inducible beta-lactamase expression and efflux pump activity [7, 11].

The molecular results demonstrated clear differences in the gene detection profiles of the two species. In *E. coli*, detection of TEM, SHV, CTX and OXA is consistent with acquired beta-lactamase-associated resistance patterns described in Enterobacterales. CTX detection was significantly more frequent in *E. coli* than in *P. aeruginosa* in the PCR-screened subset. In *P. aeruginosa*, detection of SHV, CTX, OXA, KPC, and IMP is consistent with the organism's capacity to harbour acquired beta-lactam resistance determinants, including carbapenemases. The detection of IMP in 12% (3/25) of *P. aeruginosa* isolates is notable because IMP enzymes are class B metallo-beta-lactamases with carbapenemase activity and have been described among carbapenem-resistant Gram-negative pathogens, including *P. aeruginosa* [10, 21].

However, PCR detection of IMP should be interpreted conservatively as detection of the gene target only, not as confirmation of enzyme expression, metallo-beta-lactamase phenotype or direct causation of carbapenem resistance in those isolates. Similarly, SHV and CTX findings in *P. aeruginosa* should be interpreted as detection of gene families by PCR, not as confirmation of ESBL phenotype, enzymatic activity or clinical ESBL status. ESBL phenotypic classification frameworks developed for Enterobacterales are not directly transferable to *P. aeruginosa* without organism-appropriate confirmation [7, 9].

Not all resistant isolates carried the genes screened in this study. This suggests that observed phenotypic resistance cannot be explained solely by the selected targets tested here. Other mechanisms likely contributed, including porin loss or alteration, efflux pump overexpression, altered penicillin-binding proteins and resistance genes not included in the PCR panel and not within the scope of this study or analysis. Other beta-lactamase families, including GES, VEB, PER, BEL, VIM and SPM, may also be relevant, particularly in *P. aeruginosa*, and warrant investigation in future studies [6–7, 10–11, 21].

The difference in gene distribution between the two organisms is biologically meaningful. *E. coli* typically relies more heavily on acquired ESBL-associated and plasmid-associated resistance determinants, whereas *P. aeruginosa* demonstrates a more complex resistance architecture that may combine acquired beta-lactamases with highly efficient intrinsic mechanisms. This may help explain why some *P. aeruginosa* isolates exhibited resistance even when only a limited number of screened genes were detected, and why carbapenem resistance was proportionally more prominent in *P. aeruginosa* than in *E. coli* in this dataset [6–7, 9, 11].

This study has several limitations. First, only a subset of resistant isolates underwent molecular analysis, which may limit the generalisability of gene frequency estimates to the wider resistant population. Second, although IMP was added to the carbapenemase panel, the PCR panel remained limited to selected targets and therefore could not capture the full range of beta-lactam resistance determinants present in these organisms. Third, the study did not include gene expression analysis, plasmid typing, strain typing, phenotypic metallo-beta-lactamase confirmation or whole-genome sequencing, all of which would provide greater resolution regarding resistance mechanisms, gene mobility and relatedness.

Finally, because resistance in Gram-negative organisms is often multifactorial, genotype-phenotype relationships cannot always be fully resolved using targeted PCR alone [6–11].

Despite these limitations, this study contributes important local data on the phenotypic and molecular epidemiology of beta-lactam resistance in Trinidad and Tobago. The combined detection of cephalosporin and carbapenem resistance, together with identification of multiple beta-lactamase-associated gene families across both species, including IMP-positive *P. aeruginosa* isolates, underscores the need for continued surveillance of resistant Gram-negative pathogens in the region. Future work should expand molecular testing to larger sets of isolates, include broader resistance gene panels, and incorporate sequencing-based approaches to better define the diversity, relatedness, and mobility of resistance determinants circulating locally [10, 12–14, 21].

Conclusions

This study provides a combined phenotypic and molecular assessment of beta-lactam resistance in *E. coli* and *P. aeruginosa* isolates from Trinidad and Tobago. Phenotypic testing demonstrated substantial resistance to second- and third-generation cephalosporins in both organisms, while carbapenem resistance was significantly more frequent in *P. aeruginosa* than in *E. coli*. Molecular analysis of selected resistant isolates identified distinct gene detection profiles between the two species, including CTX detection in *E. coli* and SHV, CTX, OXA, KPC, and IMP detection in *P. aeruginosa*.

These findings add to the limited published data on beta-lactam resistance in Trinidad and Tobago and highlight the complexity of resistance mechanisms in clinically important Gram-negative organisms. The detection of IMP among *P. aeruginosa* isolates supports the need to include metallo-beta-lactamase targets in future local surveillance. Continued local surveillance integrating phenotypic methods, broader molecular panels and sequencing-based approaches is needed to better define the diversity, transmission and clinical significance of beta-lactam resistance determinants circulating in the region.

Abbreviations

AMR
antimicrobial resistance
AST
antimicrobial susceptibility testing
ATCC
American Type Culture Collection
CI
confidence interval
CSF
cerebrospinal fluid
ESBL

extended-spectrum beta-lactamase
IMP
imipenemase
MBL
metallo-beta-lactamase
OR
odds ratio
PCR
polymerase chain reaction.

Declarations

Ethics approval and consent to participate

Ethics committee name: The University of the West Indies, St. Augustine, The Campus Research Ethics Committee provided ethics approval for all aspects of this study. The reference numbers from this committee for all areas of this study are as follows:

Consent for publication

This manuscript does not contain individual persons' data, images or videos.

Competing interests

The authors declare that they have no competing interests.

Authors' information

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Author Contribution

RT: conducted experimental design, performed experiments and experimental analysis, data analysis and manuscript preparationCU: Supervised project and reviewed manuscriptA.A: Performed experiments and experimental analysis, analysed data K.S: Performed experiments and experimental analysis, analysed dataN.R: Performed experiments and experimental analysis, analysed dataZ.A: Performed experiments and experimental analysis, analysed dataS.A: Performed experiments and experimental analysis, analysed dataP.E.A: Supervised project and reviewed manuscript

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Data Availability

The Raw Data generated from this study (bacterial species, antibiotic resistance profile, molecular biology PCR results) has been added as a supplementary file in the submission system of this journal

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Figures

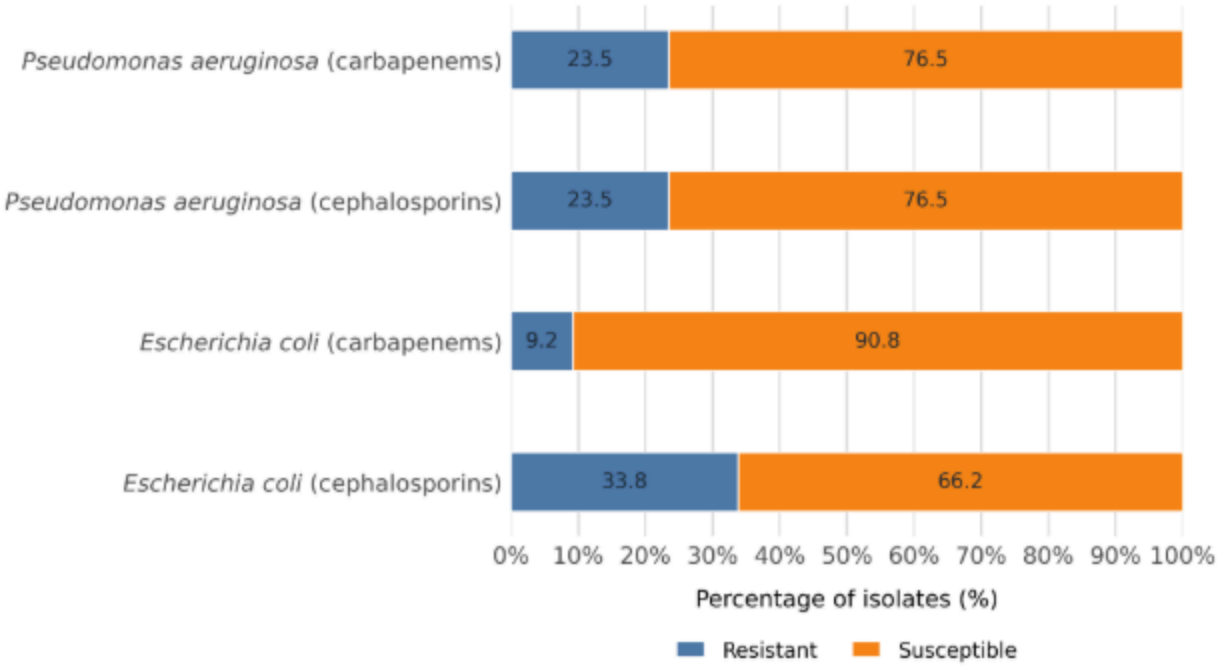


Figure 1

Resistance to cephalosporins and carbapenems in *E. coli* and *P. aeruginosa*isolates.

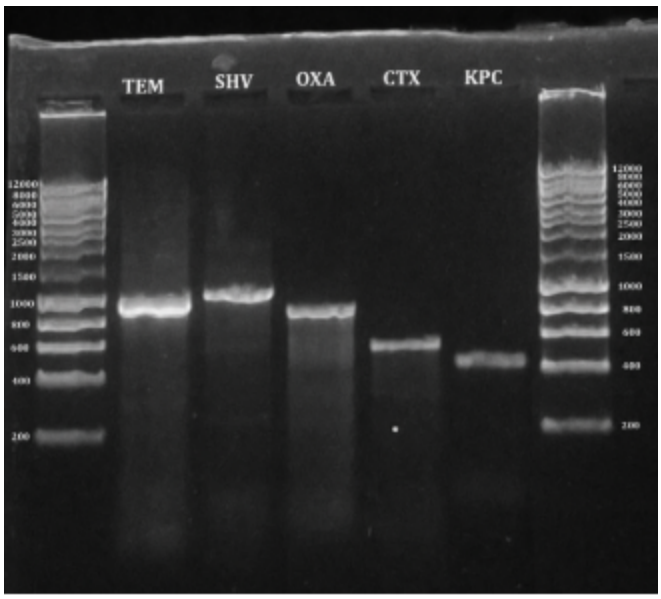


Figure 2

Representative PCR gel electrophoresis image showing several of the beta-lactamase-associated amplicons amplified. Lanes 1 and 7 represent the markers, while lanes 2 to 6 represent the different detected genes.

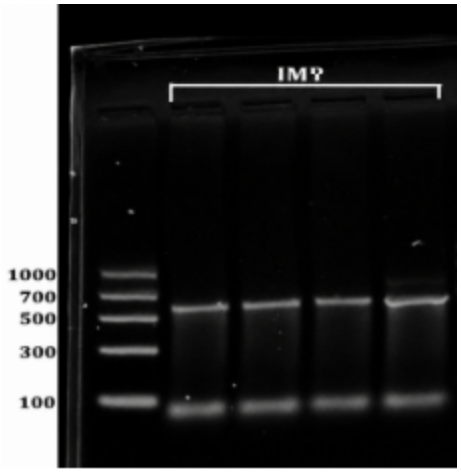


Figure 3

Representative PCR gel electrophoresis image showing the IMP metallo-beta-lactamase-associated amplicon in lanes 2 to 5; Lane 1 as the marker.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ESBLGel2merged.pdf
- BMCRawdataresults.xlsx