INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 11, Issue 6- 2024

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2024.11.06.005

Antimicrobial Susceptibility and Multidrug Resistant Patterns of *Klebsiella* species implicated in Seafood: A Study on Public Health Risks in Bayelsa State, Nigeria.

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Abstract

This study is aimed at investigating the multidrug resistance patterns of *Klebsiella aerogenes* and *Klebsiella quasipneumoniae* isolated from some seafood and understanding the genotypic mechanisms underlying these phenotypic traits with the ultimate aim to address public health risks associated with foodborne diseases. *Klebsiella aerogenes* and *Klebsiella quasipneumonia* isolated from 200 composite seafood samples consisting of periwinkle, crab, oyster and shrimps were screened for their antimicrobial susceptibility patterns to ten conventional antibiotics. The multidrug resistance patterns of each isolate was ascertained using their frequency of resistance to all the antibiotics tested. Using Real-time PCR, their multidrug resistant genes were characterized. Both *Klebsiella aerogenes* and *Klebsiella quasipneumoniae* isolated from seafood samples from both Nembe and Ox-bow lakes were all susceptible to ciprofloxacin and resistant to ampicillin, penicillin, ceftazidime and ceftriaxone. All the isolates exhibited high frequency of multidrug resistance to ceftriaxone, ampicillin, penicillin and ceftazidime. *acrAB, ctx-M, and shv* genes were implicated in the multidrug resistance exhibited by the isolates.

Keywords: Seafood, *Klebsiella aerogenes, Klebsiella quasipneumoniae*, Multidrug resistance, resistant genes.

Introduction

Given the high demand for nutrient rich diets in recent times, the consumption rate of seafood has been on the increase and studies have proven they harbor foodborne pathogens which are known to be antibiotic resistant (Krahulcová *et al.,* 2023). Other food products such as vegetables have also been identified as vehicle for animal and human foodborne pathogens (Azuwike *et al.,* 2024) Seafood obtained from rivers has higher tendencies to carry pathogens because of certain activities such as human waste discharge, industrial waste discharge and solid waste which increase the proliferation of these pathogens (Catherine *et al.,* 2021). Pathogens implicated in seafood include Enterobacteriaceae, *Staphylococcus* and *Vibrio* species and these bacteria carry antimicrobial resistant genes which contribute to antimicrobial resistance via horizontal gene transfers between and among species. Recent studies have demonstrated the effect of climate change in playing a key role in impacting disease distribution and transmission (Braide *et al,* 2020). Extreme climatic conditions lead to rivers drying up hence causing bacteria concentrations to spike and most seafood harbor these pathogens which spread by humans and animals when they consume them(Braide *et al,* 2020). Similarly, when river levels increase and flooding ensues, there is high tendency to the spread of pathogens to the environment hence facilitating the spread of antibiotic resistant species (Braide *et al,* 2020). Studies have demonstrated seafood to be an excellent vector that carries antibiotic resistant bacteria and antibiotic resistant genes making it easier to spread ARGs from food to consumers (Krahulcová *et al.,* 2023). More specifically, several studies have implicated *Klebsiella* species in both human and animal samples where they play roles as pathogens widely distributed in the environment hereby facilitating the spread of antimicrobial resistant genes (Justice-Alucho *et al.,* 2021). Antimicrobial resistance has become the world's deadliest global health challenge and the development of multidrug resistance in bacterial species has led to ineffective treatment of even the slightest infections (Wyres *et al,* 2019). Studies have reported the presence of *bla*$_{CTX-M-2}$, *bla*$_{DHA-1}$, *bla*$_{NDM}$, *bla*$_{IMP}$, *bla*$_{KHM-1}$, *bla*$_{OXA-48}$, *bla*$_{OXA-162}$, *bla*$_{OXA-10}$, *bla*$_{KPC}$, *tet*(A), and *tet(K)* genes coding for the resistance to cephalosporins, carbapenems and tetracycline in *Klebsiella* isolates from clinical and environmental (Altayb*et al.,* 2023). Multidrug resistance in *Klebsiella* species is facilitated by the spread of antimicrobial resistant genes by mobile elements via horizontal gene transfer (Suzuki *et al.,* 2019). Studies have also shown that sewage treatment plants and wastewater and anthropogenic activities are the reservoir sources of multidrug resistant genes (Akiba *et al.,* 2016). Other environmental settings harboring ARGs include water surfaces, soil, and animal waste (Sekizuka *et al*., 2018). Studies on *Klebsiella* resistance patterns and mechanisms have shown that there is an increased percentage of *Klebsiella* strains resistant to antimicrobials of the cephalosporins and fluoroquinolones groups (Wang *et al*., 2004). Resistance to fluoroquinolone is associated with mutations in the quinolone resistance-determining region of the gyrA and or parC gene coding for the target proteins DNA gyrase and topoisomerase IV, respectively (Hooper, 2000). Plasmid-mediated resistance to quinolones has also been described (Wang *et al*., 2004), and its frequency seems to be increasing in recent years (Rodríguez-Martínez *et al.,* 2003). Besides topoisomerase mutations and plasmids, altered permeability (usually because of porin loss) and energy-dependent efflux have also been shown to contribute to the fluoroquinolone resistance phenotype in *K. pneumoniae* (Martínez-Martínez *et al.,* 2002*).* One of the efflux systems involved in this resistance phenotype is multidrug efflux system in *K. pneumoniae* encoded by the *acrAB* operon. In this operon, *acrR* encodes the acrAB repressor, while *acrA* and *acrB* encode a periplasmic lipoprotein of 40 kDa, anchored to the inner membrane, that bridges the outer and inner membranes and an integral membrane protein of 113.5 kDa with 12 membrane-spanning α-helices, located in the cytoplasmic membrane, respectively (Domenech-Sanchez *et al*., 2001). The *acrB* connects with TolC, an outer membrane protein that belongs to a

family of envelope proteins found in all Gram-negative bacteria and that is essential for the expulsion of a plethora of compounds (Eswaran *et al*., 2003). *K. quasipneumoniae* have been shown to exchange chromosomal and plasmid-borne antibiotic resistance genes with other *Klebsiella* species through homologous recombination (Holt *et al*., 2015). At first, *K. quasipneumoniae* was thought to be an intestinal commensal. Nonetheless, it has been identified as an etiologic agent in several clinical *Klebsiella*-related infection cases by current genomics-driven research (Long *et al*., 2017). Long exposure of certain pathogens such as *Klebsiella* species to antibiotics in the healthcare may lead to antibiotic resistance hence it is important that antibiotics are administered only when necessary, in order to reduce the prevalence of antimicrobial resistance (Uzoagba *et al.*, 2019). Moreover, *K.quasipneumoniae* uptakes antimicrobial resistant genes and plasmids for other groups of bacteria from the enterobacteria as well as those belonging to those of the incompatible groups such as IncU/IncX5 which harbors $bla_{KPC,}$ IncH12 which harbours *mcr-9,* and IncFII/IncFIB which harbors *mcr-8.2* (Mathers *et al.*, 2019).*Klebsiella aerogenes* formerly known as *Enterobacter aerogenes* have close relation to *Klebsiella pneumoniae* as demonstrated by comparative bacterial phylogenetics via whole genome sequencing (Sutton *et al*., 2018).

This study evaluates the antimicrobial susceptibility and multidrug resistant patterns of *Klebsiella quasipneumoniae* and *Klebsiella aerogenes* isolated from selected seafood with the aim of understanding the underlying genetic mechanisms responsible for their resistance to selected antibiotics. By understanding these patterns and identifying the genes responsible, this study provides insight that would help to curb the spread of antimicrobial-resistant bacteria in food thereby contributing to efforts necessary to mitigate public health risk through targeted antimicrobial therapy, food safety and improved environmental health via the "one health approach".

Materials and Methods

Klebsiella aerogenes and *Klebsiella quasipneumoniae* previously isolated and characterized in seafood samples from a study by Justice-Alucho *et al.* (2021) were analyzed for their antimicrobial susceptibility and multidrug resistance patterns. A total of 55 *Klebsiella* species consisting of *Klebsiella aerogenes* (n=20) and *Klebsiella quasipneumoniae* (n=35) were obtained from seafood from Nembe and OX-bow lake. The isolates were resuspended in tryptic soy broth and re-streaked onto tryptic soy agar twice before carrying out the analysis (Venggadasamy, *et al.*, 2021).

Antimicrobial Susceptibility Testing

Kirby bauer disc diffusion method as described by the Clinical and Laboratory Standard Institute (2016) was used to determine the antimicrobial susceptibility patterns of the *Klebsiella* isolates to 10 conventional antibiotics including Ciprofloxacin (5ug), Tetracycline (30ug), Ceftriaxone (30ug), Erythromycin (15ug), sulfonamide/Trimethoprim (25ug), gentamicin (30ug), Ampicillin (30ug) penicillin (15ug), Streptomycin (15 ug) and ceftazidime (30ug). These antibiotics were chosen because of their relevance in the clinical setting and their use as first line drugs in the case of a foodborne disease. Pure cultures of the isolates were made by streaking discrete colonies to fresh agar medium to obtain 24-hour old cultures. The cultures were adjusted to an OD of 0.5 which is equivalent to 5 x 10^8 CFU/ml by adding the isolates to 5ml of 0.85% saline (CLSI, 2016). The cultures were 10-fold serially diluted three times to obtain a final cell concentration of 1 x 10^5 CFU/ml (CLSI, 2016). The cultures were seeded onto Mueller Hinton agar platesand antibiotic impregnated discs placed at equidistantly on the bacteria seeded with the cultures (CLSI, 2016). This was done in triplicates and the plates were incubated for 24 hours at 37^0 C and the diameter of the "zone of inhibition" was measured and interpreted

using the CLSI table which enabled classification of the isolates as either resistant or susceptible (CLSI, 2016).

Molecular characterization of antibiotic resistance genes.

DNA extraction and quantification was performed using the boiling method and Nanodrop respectively as described by (Tamura *et al.*, 2013).

shv genes from the isolates were amplified using the *shv* F: 5' CGCCTGTGTATTATCTCCCT-3' and *shv* R: 5'-CGAGTAGTCCACCAGATCCT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV(Carter *et al.*, 2010).

ctx-M genes from the isolates were amplified using the *ctx-M*F:5'-CGCTTTGCGATGTGCAG-3' and *ctx-M* R: 5'-ACCGCGATATCGTTGGT-3'primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator(Carter *et al.*, 2010).

acrAB genes from the isolates were amplified using the *acrAB*F: 5'-ATCAGCGGCCGGATTGGTAAA-3'and *acrAB*R:5'- CGGGTTCGGGAAAATAGCGCG-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator(Carter *et al.*, 2010).

The *sxt* genes of the isolates were amplified using the *sxt1* F: AGCGATGCAGCTATTAATAA and *sxt1* R: GAAGAGTCCGTGGGATTACG primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a blue light transilluminator (Carter *et al.*, 2010).

Results

Antimicrobial susceptibility Patterns of bacterial isolates in seafood from Nembe

A total of 200 bacteria species were previously isolated and characterized from periwinkle, crab, oyster and shrimp samples using the traditional microbiological method and PCR. Their susceptibility patterns were assessed as the isolates were all subjected to antimicrobial susceptibility testing by Kirby Bauer disc diffusion method to determine their susceptibilities to Ciprofloxacin (5ug), Tetracycline (30ug), Ceftriaxone (30ug), Erythromycin (15ug), sulfonamide/Trimethoprim (25ug), gentamicin (30ug), Ampicillin (30ug) penicillin (15ug), Streptomycin (15ug), and ceftazidime (30ug). The isolates were classified as resistant or susceptible using the CLSI standard by comparing the zone of inhibition with the standard values. As shown in figure 1, all the isolates n=80 (100%) from seafood samples from Nembe were susceptible to Ciprofloxacin(C) i.e none of the isolates from the different seafood samples was resistant to Ciprofloxacin. Also, no isolate from periwinkle was resistant to gentamicin but 15%, 10% and 15% of the isolates from crab, oyster and shrimp respectively were resistant to gentamycin. 25% of the isolates from periwinkle and shrimps were resistant to Erythromycin while 10% and 20% of isolates from crab and oysters respectively were resistant to it. 25% each of the isolates from periwinkle and crab were resistant to Sulfonamide/Trimethoprim while 35% each of the isolates from oysters and shrimps were resistant to it. 55% each of the isolates from crab and shrimps were resistant to tetracycline while 50% and 60% of the isolates from periwinkle and oyster respectively were resistant to it. Again, 55% of the isolates from oyster and shrimp were resistant to streptomycin while 60% of the isolates from periwinkle and crab were resistant to it as shown in Figure 1

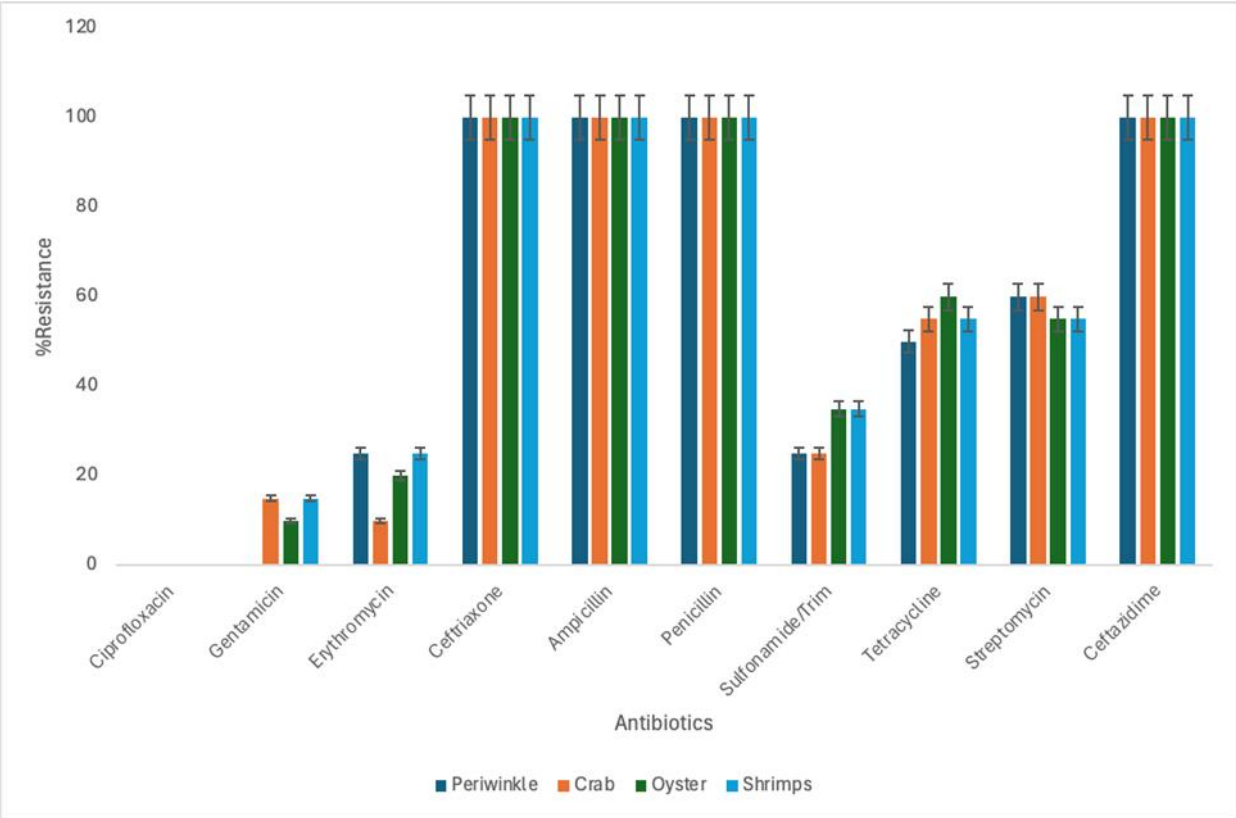

Figure 1: Susceptibility pattern of all isolates from the seafood samples from Nembe River to the antibiotics.

Antimicrobial susceptibility Patterns of bacterial isolates in seafood from Ox-bow Lake

The antimicrobial susceptibility test carried out on all isolates n=120 obtained from seafoods obtained from Ox-bow Lake showed that all the isolates were resistant to Penicillin, Ampicillin, Ceftriaxone and Ceftazidime as shown in figure 2. However, all the isolates (100%) were susceptible to Ciprofloxacin(C) i.e none of the isolates from the different seafood samples was resistant to Ciprofloxacin. Also, no isolate from crab and shrimp was resistant to gentamicin but 6.7%, and 10% of the isolates from periwinkle and oyster respectively were resistant to gentamycin. 6.7% and 20% of the isolates from periwinkle and crab respectively were resistant to Erythromycin while 10% of isolates each from shrimp and oysters were resistant to it. 26.7% of the isolates from periwinkle, 33.3% from crab, 23.3% from oysters and 13.3% of the isolates from shrimps were resistant to Sulfonamide/Trimethoprim. Furthermore, 53.3% each of the isolates from periwinkle and crab were resistant to tetracycline while isolates from oysters and shrimps showed close resistance at 33.3% and 30% respectively. Again, 63.3% of the isolates from periwinkle and 50% of the isolates from oysters were resistant to streptomycin while 30% of the isolates from crab were resistant to it and 23.3% from shrimps as shown in Figure 2.

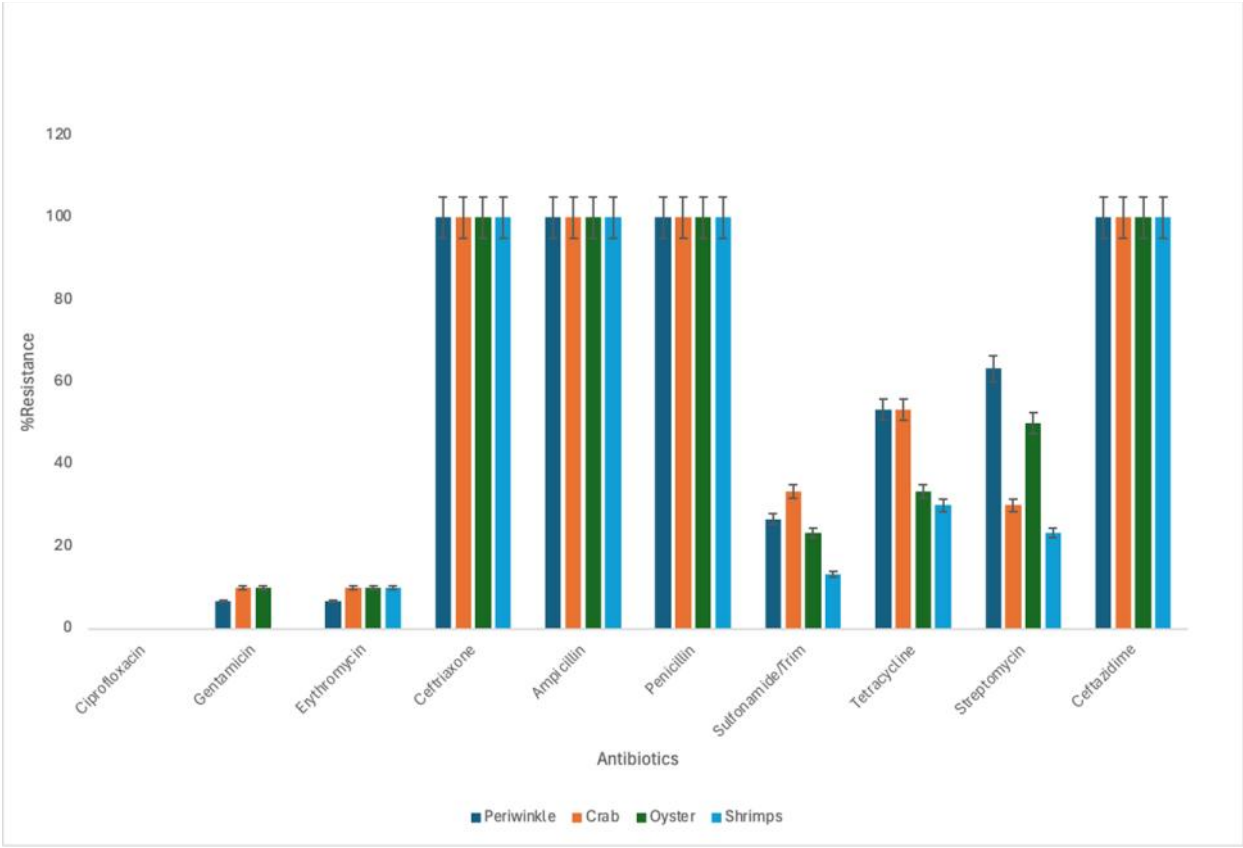

Figure 2: Resistance patterns of all isolates from the seafood samples from Ox-bow Lake to the antibiotics.

Antimicrobial susceptibility Patterns of *Klebsiella* species in seafood

Of the 200 isolates characterized in the previous study, 55 were identified as *Klebsiella aerogenes* n=20 and *Klebsiella quasipneumoniae* n=35. To better understand the resistant patterns of the *Klebsiella* species isolated from seafood samples, the number of resistant isolates was determined for each sampling point to ascertain if any overlap exists between the two species isolated. For *Klebsiella species* isolated from samples collected from Nembe, both species were all (100%) susceptible to ciprofloxacin while being resistant to ampicillin, penicillin, ceftriaxone and ceftazidime. All *Klebsiella aerogenes* (100%)were susceptible to ciprofloxacin and gentamicin and resistant to ceftriaxone, ampicillin, penicillin and ceftazidime. Similarly, all *Klebsiella quasipneumoniae* isolated were all susceptible to ciprofloxacin only. 88.2% of *Klebsiella quasipneumoniae* isolated were resistant to tetracycline and streptomycin. 5.9%, 11.8% and 35.3% of the *Klebsiella quasipneumoniae* isolated were resistant to erythromycin, gentamicin and sulfonamide/Trimethoprim respectively as shown in figure 3. For isolates from OX-bow lake, both species were all (100%) susceptible to ciprofloxacin and gentamicin while only *K. aerogenes* recorded 100% resistance to erythromycin and sulfonamide/Trimethoprim as shown in figure 4. Consistent with results obtained from Nembe, all isolates of both species were 100% resistant to ceftriaxone, ampicillin, penicillin and ceftazidime. Only 60% and 50% of the *K. aerogenes* isolates were resistant to tetracycline and streptomycin respectively. Same percentage of *K.quasipneumoniae isolates were* resistant to both sulfonamide/Trimethoprim and streptomycin while only 11.1% and 55% of the isolates were resistant to erythromycin and tetracycline respectively as represented in Figure 4.

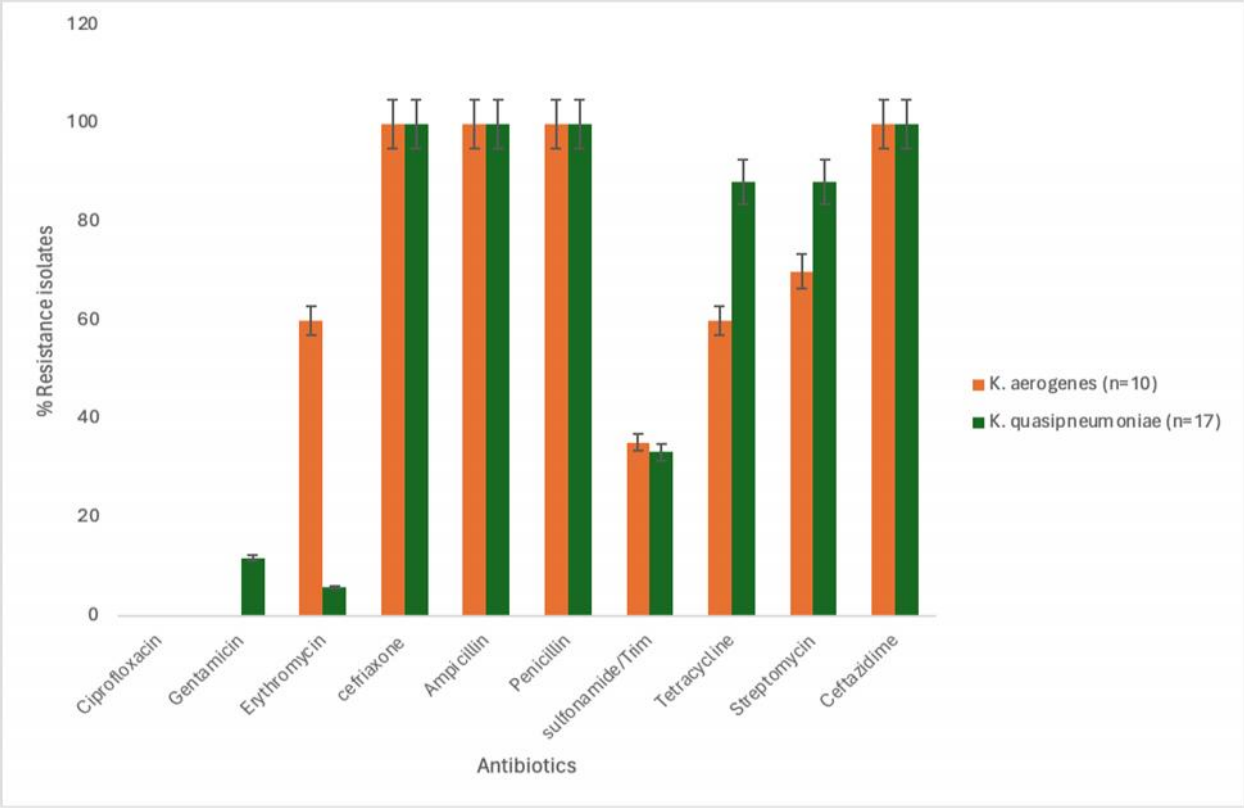

Figure 3: Resistant patterns for both *Klebsiella* species isolated from seafood from Nembe. No significant difference exists between the resistance frequency of *K quasipneumoniae* and *K. aerogenes* to ceftriaxone, ampicillin, penicillin,sulfonamide/Trimethoprim and ceftazidime unlike other antibiotics ($p<0.05$).

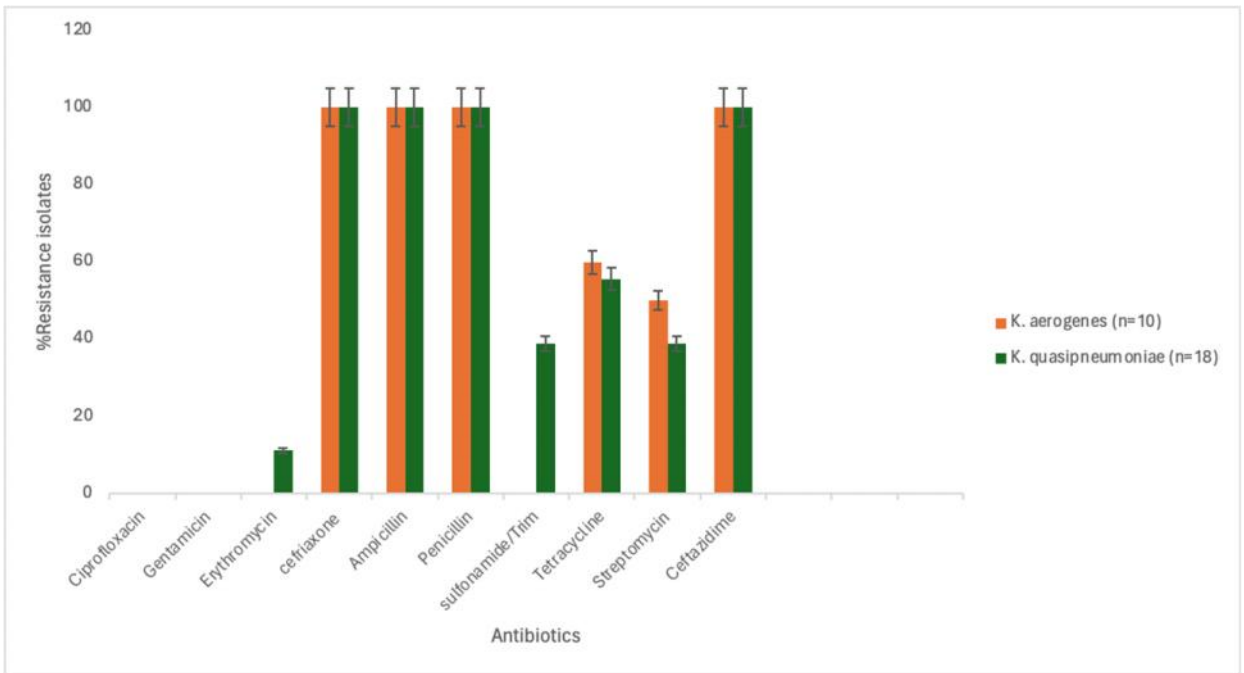

Figure 4: Resistant patterns for both *Klebsiella* species isolated from seafood from Ox-bow Lake. No significant difference exists between the resistance frequency of *K quasipneumoniae* and *K. aerogenes* to ceftriaxone, ampicillin, penicillin, tetracycline and ceftazidime unlike other antibiotics unlike the other antibiotic types ($p<0.05$).

Multidrug Resistant patterns of *Klebsiella* species in seafood.

To determine the multiple drug resistant patterns of each *Klebsiella* specie isolated from the seafood, the isolates were analyzed for their ability to resist more than one of the antibiotics used in this study utilizing the method adopted from Krumperman *et al.*(1983). 50% of the *K. aerogenes* isolated from seafood gotten from Nembe were multidrug resistant to eight (8) of the antibiotics used in this study. The highest resistance percentage frequency was to Erythromycin, Ceftriaxone, Ampicillin, Penicillin, Tetracycline, Sulfonamide/Trimethoprime, Streptomycin and Ceftazidime as shown in table 1. 5.88% of *K. quasipneumoniae* isolated from the seafood obtained from Nembe weremultidrug resistant to nine (9) antibiotics including Gentamycin, Erythromycin, Ceftriaxone, Ampicillin, Penicillin, Tetracycline, Sulfonamide/Trimethoprime, Streptomycin and Ceftazidime as shown in table 2. 52.94% of *K. quasipneumoniae* were multidrug resistant to Ceftriaxone, Ampicillin, Penicillin, Tetracycline and Ceftazidime as shown in table 2. 50% of the *Klebsiella aerogenes* isolated from seafood from Ox-bow Lake were multidrug resistant to six (6) antibiotics at a time including Ceftriaxone, Ampicillin, Penicillin, Tetracycline, Streptomycin and Ceftazidime as shown on table 3. The lowest percentage frequencies (10%) were recorded formultidrug resistance of *Klebsiella aerogenes* to Ceftriaxone, Ampicillin, Penicillin, Tetracycline and Ceftazidime as shown in table 3. 44.44% of *Klebsiella quasipneumoniae* isolated from seafood from Ox-bow Lake were multidrug resistant to Ceftriaxone, Ampicillin, Penicillin, Tetracycline and Ceftazidime as shown on table 4. The lowest percentage frequency (11.11%) was recorded for *Klebsiella quasipneumoniae* resistance to eight antibiotics including Ceftriaxone, Ampicillin, Penicillin, Tetracycline, sulfonamide/Trimethoprime, streptomycin, Erythromycin and Ceftazidime as shown in Table 4.

Table 1:Multi-Drug Resistance Pattern of *Klebsiella aerogenes (*n=10) from Seafood Samples in Nembe.

ANTIBIOTICS	FREQUENCY (%)
E+CRO+AMP+P+SXT+T+S+CAZ	50
E+CRO+AMP+P+T+S+CAZ	10
CRO+AMP+P+S+CAZ	10
CRO+AMP+P+CAZ	30

Key: E=Erythromycin, CRO= Ceftriaxone, AMP=Ampicillin, P=Penicillin, SXT= sulfonamide/Trimethoprim, T=Tetracycline, S=Streptomycin, CAZ=Ceftazidime

Table 2: Multi-Drug Resistance Patterns of *Klebsiella quasipneumoniae* (n=17) from Seafood Samples in Nembe.

ANTIBIOTICS	FREQUENCY (%)
CN+E+CRO+AMP+P+SXT+T+S+CZ	5.88
CN+CRO+AMP+P+SXT+T+S+CAZ	5.88
CRO+AMP+P+SXT+T+S+CAZ	23.53
CRO+AMP+P+T+CAZ	52.94
CRO+AMP+P+CAZ	11.76

Key: E=Erythromycin, CRO= Ceftriaxone, AMP=Ampicillin, P=Penicillin, SXT= sulfonamide/Trimethoprim, T=Tetracycline, S=Streptomycin, CAZ=Ceftazidime

Table 3: Multi-Drug Resistance Patterns of *K. aerogenes* (n=10) from Seafood Samples in Ox-Bow Lake

ANTIBIOTICS	FREQUENCY (%)
CRO+AMP+P+T+S+CAZ	50
CRO+AMP+P+T+CAZ	10
CRO+AMP+P+CAZ	40

Key:CRO=Ceftriaxone, AMP=Ampicillin, P=Penicillin, T=Tetracycline, S=Streptomycin, CAZ=Ceftazidime

Table 4: Multi-Drug Resistance Patterns of *K. quasipneumoniae* (n=18) from Seafood Samples in Ox-Bow Lake

ANTIBIOTICS	FREQUENCY (%)
E+CRO+AMP+P+SXT+T+S+CAZ	11.11
CRO+AMP+P+SXT+T+S+CAZ	27.78
CRO+AMP+P+T+CAZ	16.67
CRO+AMP+P+CAZ	44.44

Key:E=Erythromycin, CRO=Ceftriaxone, AMP=Ampicillin, P=Penicillin, SXT= sulfonamide/Trimethoprim, T=Tetracycline, S=Streptomycin, CAZ=Ceftazidime

Resistant Gene Amplification

To understand the genetic mechanisms underlying the resistance of the isolates to the antibiotics, DNA samples isolated from the isolates were analyzed for the presence of the following genes; *acrAB*, *ctx-M*, and *shv*, using real time PCR using specific primers. The primers for each gene were used to amplify the specific gene regions using PCR and they were resolved by Agarose Gel Electrophoresis and the bands were detected. *acrAB* genes were detected in *Klebsiella aerogenes* and *Klebsiella quasipneumoniae* as shown in Figure 5. The *ctx-M* genes were resolved at 500bp on the Agarose Gel Electrophoresis. The *ctx-M* genes were identified in *Klebsiella quasipneumoniae* only while absent *Klebsiella aerogenes* shown in Figure 6. The *shv* genes were resolved at 200bp on the Agarose Gel Electrophoresis. The presence of the bands shows that the gene was present in *Klebsiella aerogenes*, and *Klebsiella quasipneumoniae* as shown in Figure 7.

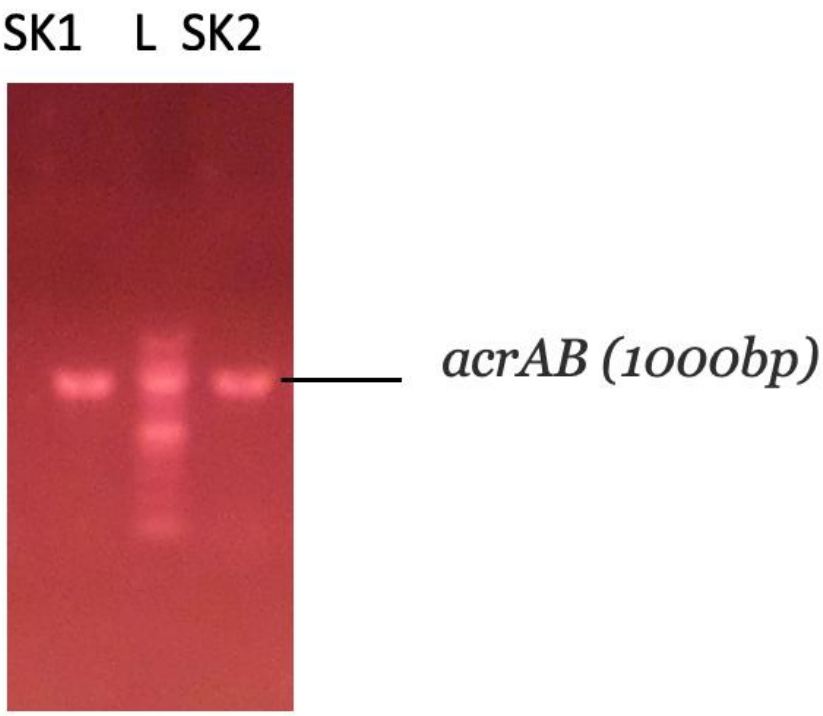

Figure 5: Bands showing acrAB genes on lanes SK1 and SK2 representing the acrAB genes at 1000bp while lane L represents the 100bp molecular ladder

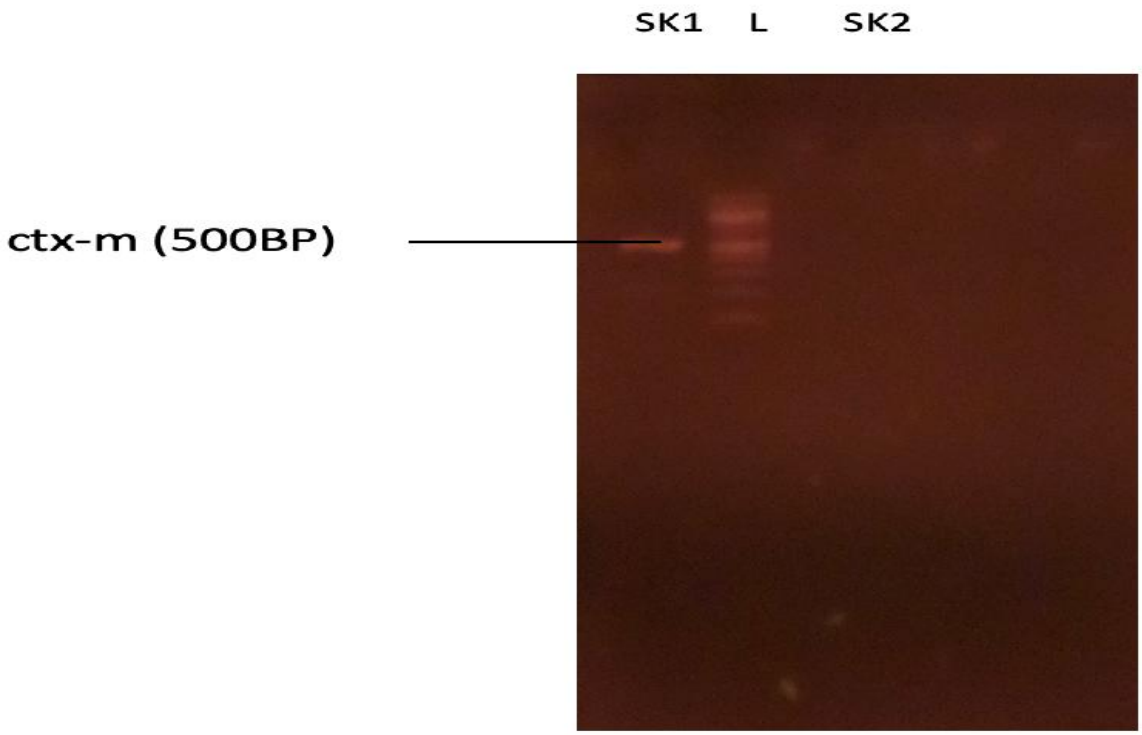

Figure 6: Bands showing ctx-m genes on lanes SK1 and SK2 representing the ctx-M genes at 500bp while lane L represents the 100bp molecular ladder.

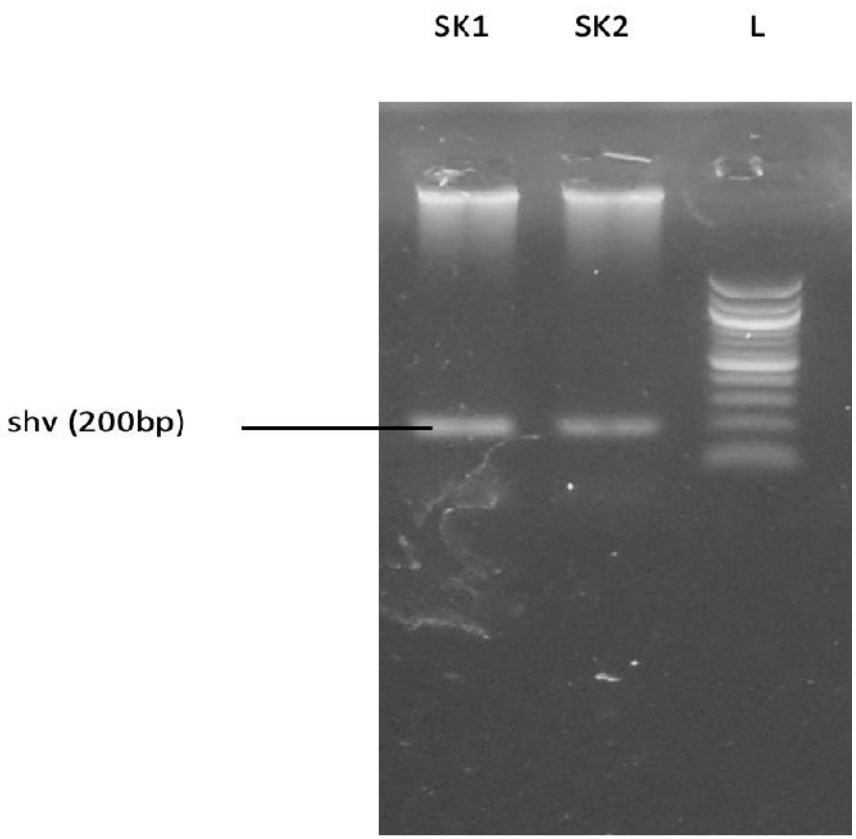

Figure 7: Bands showing shv genes on lanes SK1 and SK2 representing the shv genes at 200bp while lane L represents the 100bp molecular ladder.

Discussion

The characterization of *shv, acrAB* and *ctx-M* genes in *Klebsiella* isolates through PCR using the targeted primers underscores their role in antimicrobial resistance: The *ctx-M* genes were identified in *Klebsiella quasipneumoniae* only however, it was absent in *Klebsiella aerogenes*. The *ctx-M* gene is responsible for the resistance of the *Klebsiella quasipneumoniae*to the third generation cephalosporins which include ceftriaxone and ceftazidime used in this study (Tawfik *et al.*, 2011). It is also responsible for the resistance of *Klebsiella* to β-Lactam antibiotics used in this study corroborating reports from Tawfik *et al.*, 2011. ***shv***was also identified in *Klebsiella aerogenes* and *Klebsiella quasipneumoniae* and is responsible for their resistance to cephalosporins; ceftriaxone and ceftazidime used in this study and their resistance to β-lactam antibiotics; ampicillin and penicillin. This corroborates with reports from Tawfik *et al.*(2011).

shv (Sulfhydryl Variable) and *ctx-M* (Cefotaximase-Munich) Beta-Lactamases are extended-spectrum beta-lactamases (ESBLs) that hydrolyze a broad range of beta-lactam antibiotics, including ceftazidime, ampicillin, ceftriaxone, and penicillin Tawifik *et al.*(2011). This was confirmed by reports from Hala *et al.*(2019) who reported that in addition to the ESBLs, the presence of blaKPC-2 in *Klebsiella quasipneumoniae*, which confers resistance to carbapenems, a last-resort class of antibiotics is a huge challenge (Hala *et al.*, 2019). This gene's presence further complicates treatment options and underscores the importance of vigilant monitoring. These genes are prominent in *Klebsiella* species and contribute significantly to their multidrug resistance profile (Martinez-Romero *et al.*, 2018). In this study, the presence

of *shv* and *ctx-M* genes was specifically detected in *Klebsiella quasipneumoniae* and *Klebsiella aerogenes.* These genes render the bacteria resistant to multiple beta-lactam antibiotics, necessitating alternative therapeutic strategies (Hala *et al.*, 2019). *acrAB* genes were detected in *Klebsiella aerogenes* and *Klebsiella quasipneumoniae* and these genes are responsible for the multidrug resistance of *Klebsiella* species isolated to tetracycline, penicillin, ampicillin and erythromycin used in this this study. The *acrAB* genes encode components of the AcrAB-TolC efflux pump system, which extrudes a wide variety of antibiotics out of bacterial cells, thus contributing to multidrug resistance (Martinez-Romero *et al.*, 2018). These genes were identified in both *Klebsiella quasipneumoniae* and *Klebsiella aerogenes*, suggesting their role in reducing intracellular antibiotic concentration and enhancing resistance. This corroborates with reports by Martinez-Romero *et al*. (2018) highlighting the dual role of the *acrAB* efflux pump not only in antimicrobial resistance but also as a potential virulence factor facilitating bacterial evasion from host immune defenses, particularly in the lungs, leading to pneumonia (Martinez-Romero *et al.*, 2018).

Klebsiella species used in this study demonstrated resistance to a spectrum of antibiotics, including ceftazidime, ampicillin, ceftriaxone, and penicillin, consistent with the presence of ESBLs and *acrAB* genes. The findings from this study indicate that seafood can be a reservoir for multidrug-resistant *Klebsiella* and pose significant health risks. The multidrug resistant phenotype, bolstered by efflux pumps like *acrAB*, presents challenges for treatment and infection control. Continuous surveillance, molecular characterization, and stringent antibiotic stewardship are essential to mitigate the spread of resistant strains.

Conclusion

This study underscores the critical role of molecular techniques in accurately identifying resistance genes and understanding the complex mechanisms of antimicrobial resistance in *Klebsiella* species. This knowledge is crucial for developing effective strategies to combat multidrug-resistant infections and to conscientize the public about the health risks associated with consuming undercooked/uncooked seafood.

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Quick Response Code

DOI: 10.22192/ijcrcps.2024.11.06.005

How to cite this article:

Justice-Alucho, C. H.,Kalu, U. C., Akien-Alli, J and Braide, W. (2024). Antimicrobial Susceptibility and Multidrug Resistant Patterns of *Klebsiella* species implicated in Seafood: A Study on Public Health Risks in Bayelsa State, Nigeria. Int. J. Curr. Res. Chem. Pharm. Sci. 11(6): 28-41.
DOI: http://dx.doi.org/10.22192/ijcrcps.2024.11.06.005

