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RESEARCH ARTICLE

Bacterial Contamination Levels in Fresh Fish Fillets Sold in Lusaka District of Zambia

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Abstract

Zambia's aquaculture sector has expanded rapidly, increasing the risk of bacterial zoonotic diseases from fish. This cross-sectional study assessed bacterial contamination in 132 fresh fish fillets - 69 hake and 63 tilapias - sold in Lusaka District, Zambia, addressing gaps in local fish contamination and antimicrobial resistance. Bacterial isolates were identified through morphological characteristics and biochemical tests, while antibiotic susceptibility was determined using the Kirby–Bauer disc diffusion method. Total viable count (TVC) and faecal coliform analysis showed that 31% of samples exceeded TVC limits, and 45% contained faecal coliforms. Notably, 93% of tilapia fillets were contaminated, while hake fillets showed no faecal contamination. *Escherichia coli* (53.8%) was the predominant bacterium, followed by *Klebsiella pneumoniae* (46.2%), *Vibrio parahaemolyticus*, and *Enterobacter*. Antimicrobial susceptibility testing revealed broad-spectrum efficacy of chloramphenicol, while *Staphylococcus* and *Serratia* species exhibited resistance to penicillin. Ciprofloxacin and doxycycline were largely effective, though one *E. coli* strain showed resistance. The high contamination levels, particularly in tilapia fillets, pose serious health risks to vulnerable populations, including children, the elderly, and immunocompromised individuals. Consumption of contaminated fish could lead to gastrointestinal illness or severe infections. These findings highlight the need for improved aquaculture practices, stricter food safety regulations, and enhanced public awareness on proper fish handling and cooking to mitigate health risks.

Key Words: Bacterial contamination, fish fillets, antimicrobial resistance, *Escherichia coli*, *Klebsiella pneumoniae*, food safety, Lusaka, aquaculture, total viable count, faecal coliforms

1.0 Introduction

Fish consumption has become an integral part of the global diet due to its nutritional value, with fish and fish products being among the most traded food items worldwide (FAO/WHO, 2020). In Zambia, the aquaculture industry has experienced significant growth, expanding by 400% in the past decade (FAO,2022). Despite this growth, the safety of fresh fish remains a critical concern, particularly in areas with limited resources. As the demand for fresh fish continues to rise, so does the need to better understand the microbial safety of these aquatic products, as bacterial contamination poses risks to public health, food security, and the economy (Siamujompa et al., 2023).

Fresh fish fillets are highly perishable and susceptible to bacterial contamination during processing, handling, and storage. Fish, initially sterile, can become contaminated during filleting, and bacterial growth can proliferate depending on storage conditions (Møretrø et al., 2016). Pathogenic microorganisms, such as *Mycobacterium* spp., *Salmonella* spp., *Vibrio* spp., and *Listeria monocytogenes*, can be introduced during various stages of the supply chain, from capture to consumer (Novotny et al., 2004). Inadequate storage, poor hygiene during processing, and improper handling and transportation are major contributors to contamination. The spoilage of fish leads to foodborne illnesses, economic losses, and the wastage of valuable protein sources. Worldwide, it is estimated that 10-12 million tons of fish are lost annually due to contamination, highlighting the importance of addressing food safety concerns in the fish supply chain (FAO, 2018).

In Zambia, particularly Lusaka District, fresh fish is a widely consumed source of protein (Songe et al., 2012). However, there has been limited research on the bacterial contamination levels of locally sold fish fillets, which limits the implementation of targeted food safety measures. Additionally, the misuse of antibiotics in aquaculture has contributed to the emergence of antimicrobial-resistant bacteria, further complicating public health efforts (Cherian et al.,2023). Kimera et al., (2023) revealed that bacteria in aquaculture environments exhibit high resistance to commonly used antibiotics, such as tetracycline and ampicillin, with residues found in fish products. This presents a significant risk to human health as resistant pathogens can be transferred to consumers (O'Neill, 2014).

This study was conducted to assess the levels and types of bacterial contamination in fresh fish fillets sold in Lusaka District, Zambia by identifying the bacterial species present, determining their prevalence, and evaluating the antibiotic susceptibility profiles of the isolates. By investigating bacterial contamination and antimicrobial resistance in local fish products, this study contributes valuable data to improve food safety practices in Zambia's fish supply chain. The findings are expected to inform policy changes and guide the development of safer practices to mitigate contamination and reduce health risks. Furthermore, the results will be significant for public health authorities, food safety experts, and industry stakeholders in addressing foodborne diseases, economic losses, and antimicrobial resistance in Zambia's growing fish industry.

2.0 Materials and Methods

2.1 Study Design, Site, and Population

This cross-sectional study aimed to assess bacterial contamination in fresh fish fillets sold in retail outlets in Lusaka, Zambia. The study targeted six major retail locations: Longacres, Woodlands, East Park, Levy Mall, Twin Palm Mall, and Lewanika Mall. These locations were selected based on their high foot traffic and the availability of fish fillets, particularly in higher-end retail outlets, where fish fillets are more commonly sold. These sites ensured a representative spread of retail outlets across Lusaka, focusing on areas where the product is readily available to consumers. The study population comprised fresh fish fillets, with a focus on two species: hake and tilapia.

2.2 Sample Size and Sampling Technique

A total of 132 fish fillet samples were collected, with 22 samples obtained from each of the six selected retail locations in Lusaka District. The fish species included 69 hake and 63 tilapia fillets. The allocation between hake and tilapia was based on their relative availability at the retail outlets at the time of sampling, ensuring near-equal representation. Random sampling was employed by selecting fish fillets without prior knowledge of species or quality, using a simple random sampling approach at each outlet to minimize selection bias and provide an unbiased representation of the fish fillets sold.

2.3 Data Collection

This study was conducted between June and July 2023. Samples were collected using sterile polythene bags to prevent contamination and were transported in cooler boxes containing ice packs to maintain the integrity of the microbial load. The bacteria present in the samples were cultured on nutrient agar, and antibiotic susceptibility testing was conducted using the Kirby-Bauer disc diffusion method. The antimicrobial agents used included Penicillin-G, Amoxiclav, Amoxicillin, Cefotaxime, Ciprofloxacin, Chloramphenicol, Tetracycline, Doxycycline, and Co-trimoxazole, selected based on their efficacy against Gram-positive and Gram-negative bacteria. The plates were incubated for 24 hours at room temperature, and the zones of inhibition were measured in millimetres. Each antibiotic disc was placed equidistantly on the agar surface, with no more than five discs per plate to avoid overlapping zones. The results of the antibiotic susceptibility testing were categorized as susceptible, intermediate, or resistant following the Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines.

2.4 Data Analysis

The data collected, including prevalence and levels of microbial contamination, were analysed using Microsoft Excel 2013. The antibiotic susceptibility results were categorized based on the measured zones of inhibition and the CLSI guidelines

2.5 Ethical Considerations

The study adhered to ethical guidelines, with approval granted by Excellency in Research and Science (ERES) and the National Health Research Authority (NHRA) under the ethical clearance number Ref.No.2023-Mar-008. Given that the study was blind, explicit permission was not obtained from the retail outlets prior to sample collection. However, all samples were purchased from the outlets, and confidentiality was strictly maintained. Each outlet was assigned a unique code, and all collected data was securely entered and stored in encrypted files, accessible only to the principal investigator, ensuring anonymity of the stores and participants.

3.0 RESULTS

3.1 Bacterial Count

The findings from the bacterial contamination analysis of fresh fish chilled fillets revealed that bacterial levels, particularly the total viable count and the presence of *E. coli*, were high, indicating significant contamination (Figure 1). Among the 132 samples analysed, it was observed that a significant portion exceeded the acceptable standard of 10^5 for total viable count (Table 1). Of the 69 hake samples that passed the total viable count criterion by exhibiting a mean count of less than 7 in the first dilution, all showed negative results for

Enterobacteriaceae (Figure 2). Notably, among the remaining 63 samples, which were specifically of tilapia fillet, only 4 recorded zero Enterobacteriaceae counts (Figure 1). Most of these samples tested positive for the presence of *E. coli* and other bacterial species (Figure 1). The data in Figure 1 revealed that Location A exhibited the highest levels of both faecal counts and TVC, indicating substantial microbial contamination. In contrast, Location E reported the lowest levels.

Notably, Location D was excluded from this comparison as it did not have any tilapia fillets available for testing. The absence of data from Location D was marked clearly in the figure to avoid any misinterpretation.

3.2 Bacteria Isolated and Identified

Based on Figure 5, the most prevalent bacterial species in the sample was Escherichia coli, constituting 53.8% of the total bacteria identified. Following E. coli, Klebsiella pneumoniae was the next most prevalent, making up 46.2% of the sample. Vibrio parahaemolyticus and Staphylococcus aureus both contributed to a significant portion, with 9.10% and 7.60%, respectively. Klebsiella oxytoca followed closely behind, constituting 6.80% of the bacteria. Vibrio spp. accounted for 7.60%, while Enterobacter had a higher percentage at 15.2%. Pseudomonas accounted for 2.30%, while Serratia and Streptococcus spp. each made up 1.50% and 3.00% of the sample, respectively. Citrobacter freundii and unidentified bacteria both contributed to 3.00% and 14.4% of the total bacteria identified, as shown in Figure 5.

3.3 Antibiotic Susceptibility

Following the antimicrobial susceptibility test, it was observed that chloramphenicol, a broad-spectrum antibiotic, was effective against a wide range of bacterial species, including Vibrio, Klebsiella, E. coli, Streptococcus, Staphylococcus, Serratia, and Pseudomonas (Table 2). In contrast, penicillin, a narrower spectrum antibiotic, showed resistance against Staphylococcus and Serratia species. Ciprofloxacin, another broad-spectrum antibiotic, was effective against all tested bacteria, except for a specific strain of E. coli. Cotrimoxazole exhibited broad-spectrum activity against most bacteria, except for Pseudomonas and E. coli, which were resistant. Doxycycline, also a broad-spectrum antibiotic, was effective against all bacteria except for E. coli, which displayed intermediate resistance. Amoxicillin, which had a broad-spectrum profile, demonstrated activity against most bacteria, except for Citrobacter, which showed intermediate resistance, and Vibrio spp., which were resistant. Lastly, Dexamethasone, though not an antibiotic but a steroid, showed activity against all bacteria except for a specific strain of Citrobacter.

4.0 Discussion

This study This investigation examined the microbial quality of fish fillets, focusing on Nile tilapia and hake sold in retail markets in Lusaka District, Zambia. It revealed significant differences between the observed bacterial counts and the microbiological limits set by the Southern African Development Community (SADC HT 82: 2023), which prescribed a maximum permissible Total Viable Count (TVC) of 10^5 CFU/g. The findings showed that 53.8% of the fish samples exceeded this threshold, with 45% contaminated by faecal coliforms.

Factors contributing to these elevated bacterial counts included inadequate hygiene and handling practices, such as improper handwashing, contamination of equipment, cross-contamination between raw and processed fish, and suboptimal refrigeration (Al-Sheraa, 2018). The initial quality and source of fish, potential exposure to polluted water, and inadequate storage or processing conditions also played significant roles (Møretrø et al., 2016). These results were concerning given the popularity of Nile tilapia, which had been linked to food safety risks in other studies, such as those by Onjong et al. (2018) and Sarkar (2020), who found lower bacterial counts in fresh tilapia fillets.

Furthermore, compared to the study by Al-Sheraa (2018) in Saudi Arabia, where locally cultured fish showed better microbial quality, the findings highlighted the risks posed by imported fish, particularly due to potential contamination pre-freezing and during transportation. This aligned with findings by Aboagye et al. (2020), who noted that contamination could also occur through improper freezing techniques and handling by market sellers.

The microbial profiles of hake and tilapia differed, with hake exhibiting lower bacterial counts. Hake, a saltwater fish, benefited from advanced processing techniques and strict hygiene protocols, leading to lower microbial contamination compared to Nile tilapia, which was more prone to contamination due to its freshwater environment and handling practices (Shikongo et al., 2011; Antunes et al., 2019).

These findings emphasized the importance of enforcing better hygiene and processing standards to reduce the microbial risks associated with fish consumption in Zambia and other regions with similar supply chain challenges (Mumbo et al., 2023).

The study isolated several pathogens, including *Vibrio* spp. and *E. coli*, from apparently healthy Nile tilapia fillets. The findings contrasted with the microbiological standards outlined by SADC HT 82: 2023, which recommended the absence of these pathogens in fish products. In total, ten different bacterial genera, including *Klebsiella* spp., *Enterobacter* spp., *Citrobacter freundii*, *Pseudomonas* spp., *Staphylococcus aureus*, and *Streptococcus* spp., were identified, posing significant public health concerns.

Comparing these findings with Chitambo et al. (2023), who identified similar pathogens in eels, and Siamujompa et al. (2023), who documented pathogenic bacteria in Nile tilapia, the results highlighted widespread contamination risks. *E. coli*, a marker for faecal contamination, was dominant in the samples, confirming the significance of environmental contamination as a source of microbial load. Gufe et al. (2019) reported similar findings in Zimbabwe, linking the prevalence of *E. coli* and *S. aureus* to poor handling practices.

The presence of enteric bacteria such as *E. coli* and *Klebsiella* spp. in fish was indicative of significant environmental and faecal contamination, likely due to sewage effluent, human handling, and industrial or agricultural waste (Sheng et al., 2021). The identification of haemolytic *E. coli* raised concerns about potential diarrheal diseases in vulnerable populations. This aligned with research by Mumbo et al. (2023), which stressed the need for improved handling practices to mitigate contamination risks.

The study also noted the absence of more dangerous pathogens like *Salmonella, Listeria*, and *Shigella*, which were reported in similar studies (Al-Sheraa, 2018). However, the detection of *Staphylococcus aureus* suggested contamination from human or animal waste, either at the point of capture or during handling (Sheng et al., 2021). The widespread isolation of opportunistic bacteria such as *Streptococcus* spp. further underscored the need for better husbandry conditions to prevent disease outbreaks (Bwalya et al., 2020).

The antimicrobial susceptibility testing revealed encouraging results with most bacterial strains showing susceptibility to Chloramphenicol and Ciprofloxacin, which were commonly used to control bacterial contamination in fish. However, the resistance of E. coli to Ciprofloxacin and the intermediate susceptibility of *Klebsiella* to doxycycline raised concerns about the spread of antibiotic-resistant strains through the food chain. These results highlighted the need for ongoing monitoring and responsible antibiotic stewardship.

Similar findings were reported by Siamujompa et al. (2023), where doxycycline proved effective against most bacterial strains, except for E. coli, which displayed intermediate resistance. The resistance to Clotrimazole in *Pseudomonas* spp. and *E. coli* 1b, as well as resistance of *Vibrio* species to amoxicillin, was alarming, as *Vibrio* spp. were known to cause severe foodborne illnesses (Novoslavskij et al., 2016). This suggested the need for stronger surveillance and intervention measures to prevent the spread of antibiotic-resistant pathogens in the food chain.

The resistance patterns observed in *Staphylococcus* spp. and *Serratia* spp. aligned with global trends of rising antibiotic resistance, reinforcing the importance of judicious antibiotic use and the development of alternative treatments (Gufe et al., 2019).

E. coli remained a critical pathogen, particularly in developing regions, where it was associated with diarrheal diseases. The infectious dose for *E. coli* could be as low as 10–100 CFUs, underlining the risks posed by contaminated fish products (Ishii et al., 2008). The identification of *Klebsiella pneumoniae*, which could produce histamine and cause seafood poisoning, was of particular concern for food safety (Mohan et al., 2016). The detection of *Klebsiella* in the samples highlighted the importance of stringent food safety measures to mitigate the risks of histamine-related seafood poisoning.

In addition, *Staphylococcus* spp., *Pseudomonas* spp., and *Vibrio* species were all major foodborne pathogens, and their presence in fresh fish fillets suggested significant contamination risks. The increasing antimicrobial resistance of these pathogens emphasized the need for robust food safety management systems throughout the fish value chain (Mohan et al., 2016; Novoslavskij et al., 2016).

This study underscored the need for baseline data and early warning systems to monitor and manage zoonotic diseases in fish. As suggested by Faour et al. (2020), establishing such systems would enable proactive monitoring and intervention, reducing the risk of contamination and foodborne outbreaks. Given the diverse sources of contamination and the variety of bacteria isolated, it was evident that improved processing, handling, and regulatory enforcement were crucial to safeguarding public health.

Conclusion

The research findings indicated significant bacterial contamination of fresh fish fillets sold in Lusaka District, Zambia, particularly in tilapia. The contamination levels exceeded the microbiological limits set by SADC HT 82: 2023, with over half of the fish samples exhibiting bacterial counts above the recommended threshold.

The contamination was characterized by high levels of faecal coliforms and the predominance of pathogenic bacteria such as *Escherichia coli, Klebsiella pneumoniae*, and *Vibrio parahaemolyticus*. These pathogens presented considerable public health risks, with *E. coli* linked to diarrheal diseases*, Klebsiella pneumoniae* associated with histamine poisoning, and *Vibrio parahaemolyticus* known for causing foodborne illness.

The antimicrobial susceptibility testing revealed varying degrees of resistance among the identified bacteria, with notable resistance observed in *E. coli* and *Klebsiella pneumoniae* to multiple antibiotics. This resistance was concerning, as it highlighted the potential for antibiotic-resistant bacteria to enter the food chain, necessitating improved monitoring and regulation to safeguard public health.

These findings underscored the urgent need for improved handling, storage, and processing practices to reduce bacterial contamination in fish products. Enhanced food safety standards, coupled with stronger surveillance systems and responsible antimicrobial use, were crucial to mitigating the risks posed by bacterial pathogens in the fish supply chain in Zambia**.**

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APPENDICES

Table 1: Microbiological limits for fresh fish and chilled fish according to SADC HT 82: 2023 standards

S/N	Micro-organisms	Max. limits	Method of test
1	*Salmonella* per 25 g	Absent	ISO 6579
2	*E. coli* per gram	Absent	ISO 7251
3	*Staphylococcus aureus* cfu per gram	10^{-2}	ISO 6888
4	*Vibrio* spp per gram	Absent	ISO 21872
5	Total viable count per gram	10^{-5}	ISO 4833
6	*Listeria monocytogenes*	Absent	ISO 11290

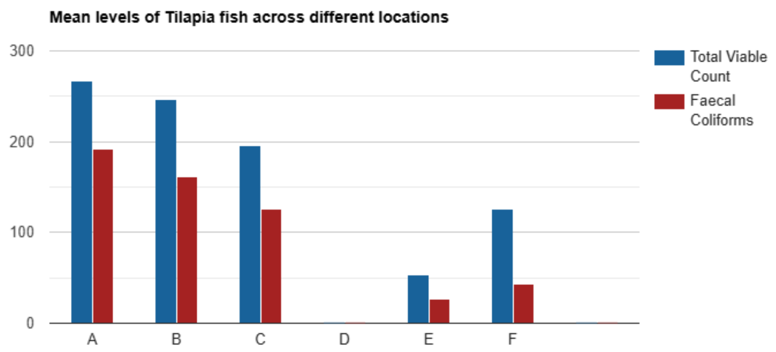

Figure 1: Mean levels of faecal and Total viable Counts in Tilapia fish across different locations.

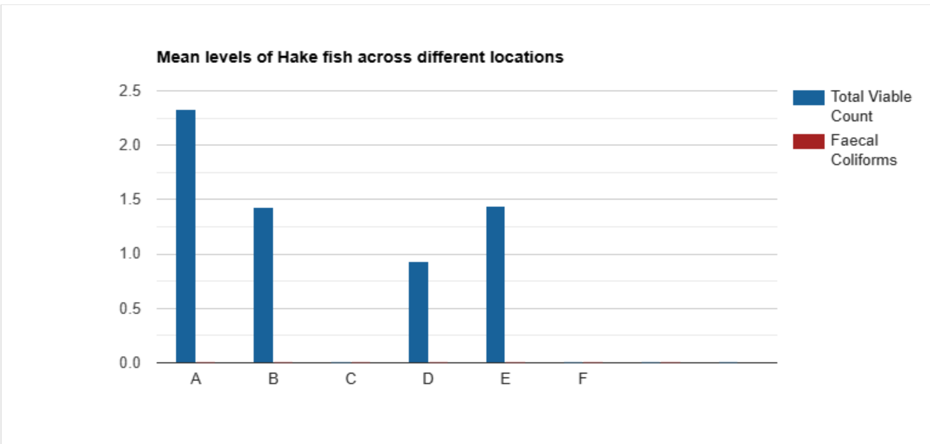

Figure 2: Mean levels of Faecal coliform and Total Viable count in Hake Fresh Fillets across different location

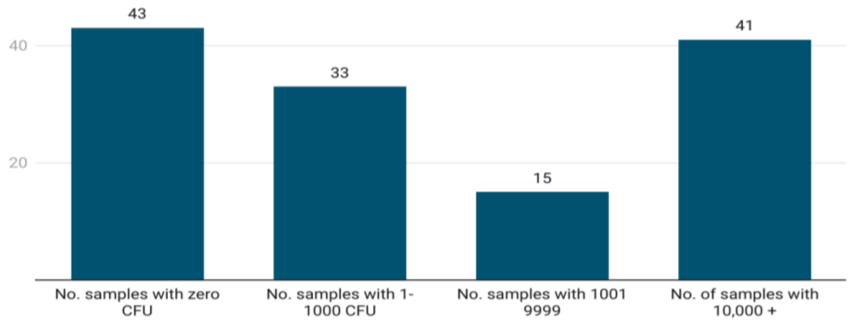

Figure 3: Average number of CFUs in collected samples for Total Viable Count

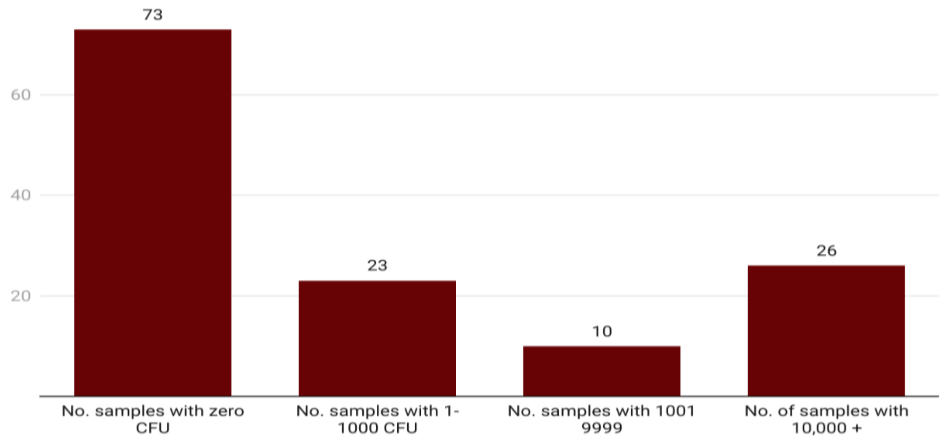

Figure 4: Average number of CFUs in collected samples for faecal coliform counts

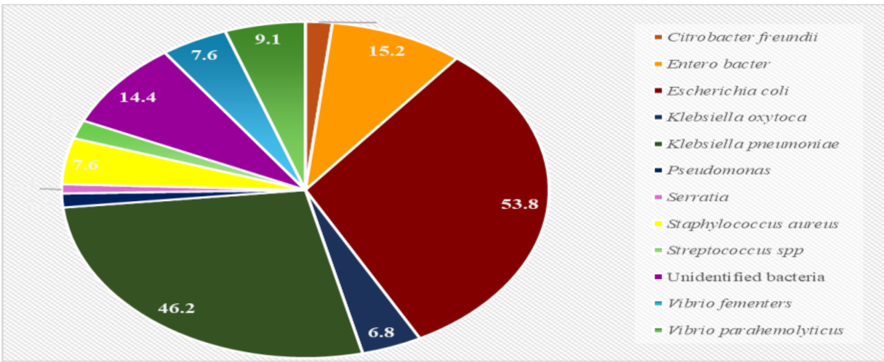

Figure 5: Prevalence of isolated bacteria from fresh fish fillet

Table 2: Antibiotic susceptibility

Bacteria	Resistant(R)	Susceptible (S)	Intermediate (I)
Vibrio non-fem	Amox	Doxy, Cotrim, Cipro, Chlor, Te	Pen, CEC, Cefotax
Citrobacter spp.	None	Doxy, Cotrim, Cipro, Chlor, Te	Amox
E. coli	Cotrim, Cipro	Amox, Doxy, Chlor, CEC	Pen, Cefotax, Te
Klebsiella spp.	None	Amox, Doxy, Cotrim, Cipro, Chlor, CEC	None
Streptococcus spp.	None	Amox, Doxy, Cotrim, Cipro, Chlor, Te	None
Staphylococcus aureus	Pen	Amox, Doxy, Cotrim, Cipro, Chlor	None
Serratia spp.	Pen	Amox, Cotrim, Cipro, Chlor	None
Pseudomonas spp.	Cotrim	Cipro, Te	Amox, Doxy, Pen, CEC, Cefotax
Vibrio fem	None	Doxy, Cotrim, Cipro, Chlor, CEC	Pen, Amox, Cefotax, Te