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Antibiotic Resistance Profile of Pathogenic Bacteria Isolated from "Mabokés" Smothered Fish in Brazzaville, Congo

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Abstract

The smothered fish samples were taken from 3 markets. They were grown on different selective and differentiated culture media to target groups of bacteria associated with food poisoning. Isolates were identified on the basis of cellular and colonial morphologies on selective and differentiated culture media, followed by susceptibility testing to certain families of antibiotics, in particular beta-lactams. This study showed that *S. aureus* and *B. cereus* had high levels of beta-lactam resistance. However, these strains were sensitive to kanamycin, tobramycin, ciprofloxacin and norfloxacin. The characteristic penicillinase phenotype was dominant in Gram-positive bacteria. *Shigella* spp, *Salmonella* spp and *E. coli* were resistant to beta-lactam antibiotics. Tobramycin and meropenem retained their activity on all strains. Despite the increased rates of resistance observed, vancomycin, kanamycin, tobramycin, gentamicin, ciprofloxacin, and norfloxacin can be used in the treatment of community-acquired infections caused by Gram-positive bacteria while meropenem and tobramycin for *Shigella* spp, *Salmonella* spp and *E. coli* infections.

Keywords

Resistance, Antibiotics, Bacteria, Smothered Fish

1. Introduction

After half a century of antibiotic use, the emergence and spread of bacterial re-

sistance are a critical public health issue. Bacterial resistance is growing in importance worldwide, especially in hospitals, and has now reached dangerous proportions in all regions of the world [1]. The extensive use of broad-spectrum antibiotics in human medicine to treat infections without diagnosis of the specific pathogen involved and in animal husbandry is considered to be a major factor in bacterial resistance to antimicrobials. Resistance can also be coupled with poor environmental conditions [2]. Indeed, infections caused by antibiotics resistant bacteria lead to high morbidity or mortality [3]. Fish is one of the most popular foods in the world population. It is a source of protein and essential oligonucleotides and is very valuable for nutritional balance [4] [5]. The diversity of cultures in the world has given rise to a wealth of recipes and methods for preparing fish. In the Republic of Congo in rural areas as well as in urban areas including Brazzaville, fresh fish is eaten in stews, sauces, barbeques, fried in oil, grilled and stewed. Fish in stews are very popular and eaten a lot. In addition to households, fish stews are prepared and then sold in markets, on public roads, in restaurants and drinking establishments, and at fish auctions by itinerant women. Due to the high consumption of these products, it is therefore necessary to ensure their hygienic quality. To date, no studies have been carried out on the antibiotic resistance of bacteria isolated from fish to smothering. Therefore, we proposed to establish the antibiotic resistance profile of pathogenic bacteria isolated in fish stewed sold and consumed in the markets of Brazzaville in order to assess the health risks related to the consumption of these products.

2. Materials and Methods

2.1. Sampling of Smothered Fish

Smothered fishes sold in Brazzaville were taken from Moukondo market (Site 1), Total market (site 2) and Moungali market (site 3). Three samples were purchased per site. At the time of purchase, the samples were placed in ice box and sent to the laboratory for analysis.

2.2. Isolation and Identification of Bacteria from Smothered Fish

In a test tube containing 9 mL of distilled water, 1 mL of the smothered fish soup was added as the stock solution from which the decimal dilutions were made. The inoculations were made on agar plate of four different culture media: 1) Mannitol egg Agar (Mannitol egg Yolk Polymixin Agar, GranuCult) for isolation of bacteria of the genus *Bacillus*, in particular *Bacillus cereus*; 2) Mannitol Salt Agar (Bio RAD) for isolation of bacteria of the genus *Staphylococcus* including mannitol positive *Staphylococcus* (*Staphylococcus aureus*); 3) *Salmonella-Shigella* Agar (Bio RAD) for isolation of bacteria of the genus *Salmonella* whose colonies are black-centered (H_2S+) and lactose-positive and the genus *Shigella* whose colonies are colorless (H_2S−) and lactose-negative; and 4) Methylene Blue Eosin Agar (Bio RAD) for isolation of enterobacteria in which the major species is *Escherichia coli* which is characterized by a metallic sheen.

The plates were incubated aerobically at 37˚C for 24 hours. Each colony was purified by separate streaking until distinct and homogeneous colonies were obtained. To ensure the purity of the bacterial isolates; microscopic observation was carried out. The isolates were identified based on cellular morphology by Gram stain and color of colonies and biochemical reaction on the growth media [6]. Isolates were stored at 4˚C in cryotubes containing 20% glycerol in LB broth.

2.3. Antibiotics *S*ensitivity Test

The antibiotics tested on *S. aureus* and *B. cereus* were: penicillin G (P., 10IU), oxacillin (OXA., 1 µg), cefoxitin (CX., 30 µg), vancomycin (VA., 30 µg), Kanamycin (K., 5IU), gentamicin (CN., 10IU), tobramycin (TOB., 10 µg), fosfomycin (FF., 200 µg), ciprofloxacin (CIP., 5 µg), norfloxacin (NOR., 10 µg), erythromycin (E., 15 µg), clindamycin (DA., 2 µg), pristinamycin (PT., 15 µg), fusidic acid (FA., 10 µg), rifampicin (RA., 5 µg). For *Salmonella spp*, *Shigella spp* and *E. coli*, the antibiotics tested were: ticarcillin (TIC., 75 µg), ticarcillin + clavulanic acid (TIM., 75 + 10 µg), piperacillin (PRL., 75 µg), piperacillin + tazobactam (TPZ., 110 µg), cefepime (CEF., 30 µg), ceftazidime (CAZ, 30 µg), aztreonam (ATM., 30 µg), Meropenem (MEM., 10 µg), Kanamycin (K., 5IU), gentamicin (CN., 10IU), tobramycin (TOB., 10 µg), fosfomycin (FF., 200 µg), ciprofloxacin (CIP., 5 µg), norfloxacin (NOR., 10 µg).

The antibiotic resistance profile of the bacterial strains was evaluated by the standard Kirby-Bauer disc diffusion method [7] [8]. The inoculum was prepared by suspending of a well-isolated colony of a young; pure bacterial culture (24 hours on agar medium) in 5 ml normal saline and the turbidity of the suspension was adjusted using spectrophotometer to 0.1 at 625 nm. The optical density of 0.1 at a wavelength of 625 nm is equivalent to 0.5 Mac Farland [9]. The culture medium, Mueller-Hinton agar, was inoculated using the swab as recommended by CLSI (Clinical and Laboratory Standard Institute) [10]. The antibiotic discs were then applied to the inoculated Mueller Hinton agar medium. The plates were incubated at 37˚C for 18 - 24 h. The diameter of bacterial growth inhibition area around the disc after incubation were measured and the antibiotics susceptibility was interpreted based on the breakpoint values published by the Antibiogram Committee of the French Society of Microbiology [11]. The strains were categorized as either: sensitive, intermediate or resistant against the antibiotics.

3. Results

3.1. Isolation and Identification of Bacterial Isolates

A total of 25 pathogenic bacteria of which 15 (60%) were gram-negative and 10 (40%) were gram-positive were isolated from the smothering fish sold in the markets of Brazzaville. **Figure 1** shows the number of bacteria isolated per site. Bacteria were mostly isolated from the samples from site 3 (Moungali market).

Figure 2 shows the repartition of the identified strains. Of these strains, 5

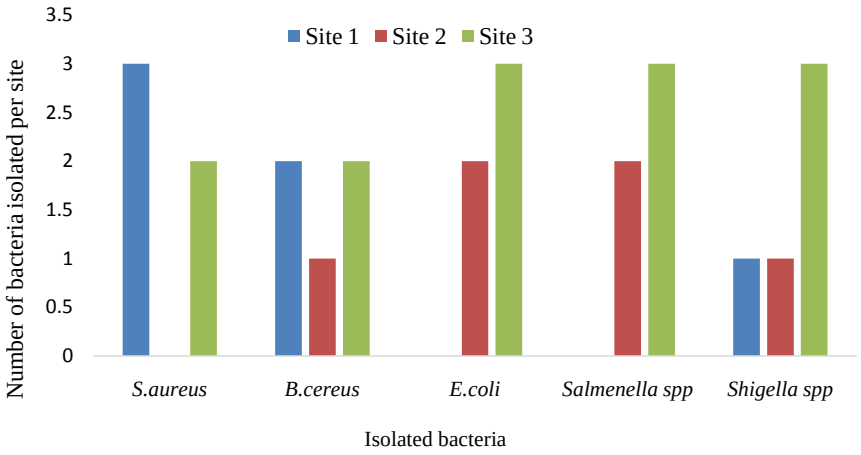

Figure 1. Number of bacteria isolated per site (Site 1: Moukondo market, Site 2: Total market, Site 3: Moungali market).

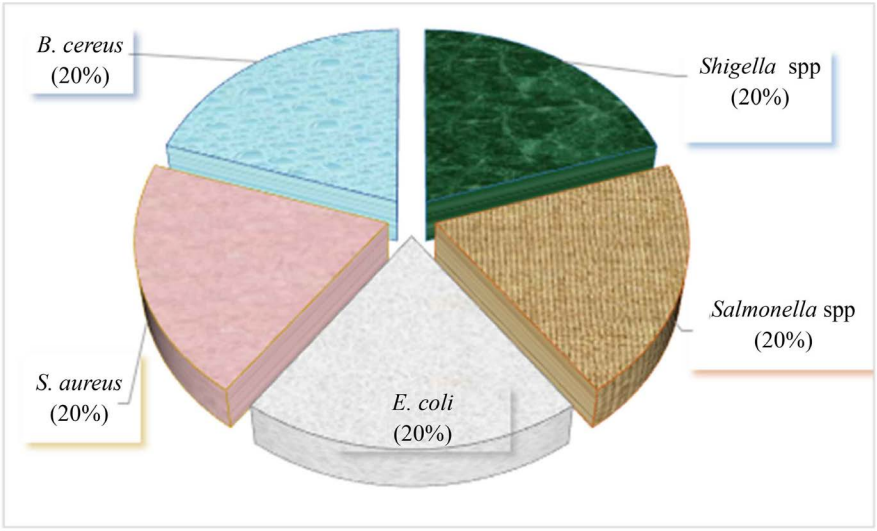

Figure 2. Frequency of identified bacteria.

(20%) were *Shigella* spp, 5 (20%) *Salmonella* spp, 5 (20%) *E. coli*, 5 (20%) *S. aureus* and 5 (20%) *B. cereus.*

3.2. Antibiotic Sensitivity Test

The results of the antibiotic sensitivity test are shown in Table 1 and Table 2. Table 1 represents the antibiotics resistance rate in *S. aureus* and *B. cereus*. These strains were resistant to beta-lactam antibiotics, which were sensitive to aminoglycosides and quinolones. Table 2 shows that *Shigella* spp, *Salmonella* spp and *E. coli* were resistant to beta-lactam antibiotics with the exception of meropenem. These strains were sensitive to tobramycin. *Salmonella* spp and *E. coli* were susceptible to quinolones.

Figure 3 shows the resistance profile of *S. aureus* and *B. cereus*. This figure shows that *S. aureus* and *B. cereus* were susceptible to kanamycin, tobramycin, ciprofloxacin and norfloxacine. High levels of resistance were observed to beta-

Table 1. Antibiotic resistance rate of *S. aureus* and *B. cereus.*

Families of antibiotics	ATB tested	*S. aureus* (n = 5)			*B. cereus* (n = 5)		
		R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Beta-lactam	P	4 (80)	0	1 (20)	4 (80)	0	1 (20)
	OX	5 (100)	0	0	5 (100)	0	0
	CX	3 (60)	0	2 (40)	5 (100)	0	0
Fosfomycins	FF	4 (80)	0	1 (20)	5 (100)	0	0
Glycopeptides	VA	1 (20)	0	4 (80)	0	0	5 (100)
Aminosides	K	0	0	5 (100)	0	0	5 (100)
	TOB	0	0	5 (100)	0	0	5 (100)
	CN	0	0	5 (100)	1 (20)	0	4 (80)
Quinolones	CIP	0	0	5 (100)	0	0	5 (100)
	NOR	0	0	5 (100)	0	0	5 (100)
MLSB	E	3 (60)	0	2 (40)	2 (40)	3 (60)	0
	DA	2 (40)	3 (60)	0	4 (100)	1 (20)	0
	PT	1 (20)	1 (20)	3 (60)	4 (80)	1 (20)	0
Fusidic acid	FA	2 (40)	0	3 (60)	0	0	5 (100)
Rifamycin	RA	3 (60)	2 (40)	0	4 (80)	1 (20)	0

ATB tested = Antibiotics tested; R = Number of resistant strains; I = Number of intermediate resistant strains; S = Number of susceptible strains; (%) = Percentage of sensitivity and resistance; MLSB = Macrolides-lincosamides-Streptogramines; P = Penicillin G; OXA = oxacillin; CX = cefoxitin; VA = vancomycin; FF = Fosfomycin; K = Kanamycin; TOB = tobramycin; CN = Gentamycin; CIP = ciprofloxacin; NOR = Norfloxacin; E = Erythromycin; DA = Clindamycin; PT = pristinamycin; FA = fusidic acid; RA = Rifampicin.

Table 2. Antibiotic resistance rates of *Shigella* spp, *Salmonella* spp and *E. coli.*

Families of antibiotic	ATB tested	*Shigella* spp (n = 5)			*Salmonella* spp (n = 5)			*E. coli* (n = 5)		
		R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Beta-lactamin	TIC	5 (100)	0	0	5 (100)	0	0	5 (100)	0	0
	TIM	5 (100)	0	0	5 (100)	0	0	5 (100)	0	0
	PRL	5 (100)	0	0	5 (100)	0	0	5 (100)	0	0
	CAZ	5 (100)	0	0	5 (100)	0	0	5 (100)	0	0
	FEP	5 (100)	0	0	5 (100)	0	0	5 (100)	0	0
	MEM	0	0	5 (100)	0	0	5 (100)	0	0	5 (100)
	ATM	5 (100)	0	0	3 (60)	2 (40)	0	5 (100)	0	0
	TPZ	5 (100)	0	0	5 (100)	0	0	5 (100)	0	0
Fosfomycin	FF	5 (100)	0	0	5 (100)	0	0	5 (100)	0	0
Aminosides	TOB	0	1 (20)	4 (80)	0	0	5 (100)	0	0	5 (100)
	CN	3 (60)	2 (40)	0	2 (40)	3 (60)	0	2 (40)	3 (60)	0
	K	4 (80)	1 (20)	0	5 (100)	0	0	5 (100)	0	0
Quinolones	CIP	5 (100)	0	0	0	0	5 (100)	5 (100)	0	0
	NOR	5 (100)	0	0	0	0	5 (100)	5 (100)	0	0

ATB tested = Antibiotics tested; R = Number of resistant strains; I = Number of intermediate resistant strains; S = Number of susceptible strains; (%) = Percentage of sensitivity and resistance; TIC = ticarcillin; TIM = ticarcillin + Clavulanic acid; PRL = piperacillin; TPZ = piperacillin - tazobatam; CAZ = ceftazidime; FEP = cefepime; MEM = meropenem; ATM = aztreonam; FF = fosfomycin; TOB = tobramycin; CN = gentamycin; K = kanamycin; NOR = norfloxacin; CIP = ciprofloxacin.

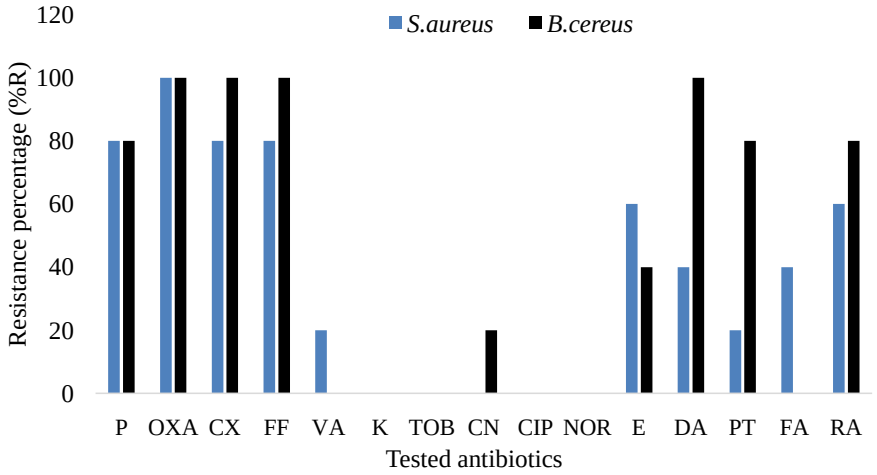

Figure 3. Antibiotic resistance profile of *S. aureus* and *B. cereus*.

lactam antibiotics. The resistance of these bacteria (*S. aureus* and *B. cereus*) to beta-lactam antibiotics was characterized by P OXA CX. The most apparent MLSB resistance phenotype was EDAPT (20%) in *S. aureus* and the least apparent in *B. cereus*. The DA PT phenotype was widely encountered in *B. cereus* (40%).

Of all the antibiotics tested, *Shigella* spp, *Salmonella* spp and *E. coli* were beta-lactam resistant apart from meropenem, which retained its activity. Tobramycin retained activity on all strains. *Salmonella* spp were susceptible to ciprofloxacin and norfloxacin (**Figure 4**). In *Shigella* spp, *Salmonella* spp and *E. coli*, only one type of beta-lactam resistance phenotype was observed: TIC TIM PRL TPZ CAZ FEP ATM. In *Salmonella* spp, 2 strains showed the CN K phenotype for aminoglycosides. The CIP NOR phenotype was widely observed in *E. coli* and *Shigella* spp.

4. Discussion

In our study, we investigated the antibiotic resistance of strains of *S. aureus*, *B. cereus*, *Shigella* spp, *Salmonella* spp and *E. coli*. The susceptibility test in *S. aureus* showed the following results: penicillin G (80%), oxacillin (100%), cefoxitin (60%), vancomycin (20%), kanamycin (0%), gentamicin (0%), tobramycin (0%), fosfomycin (80%), ciprofloxacin (0%), norfloxacin (0%), erythromycin (60%), clindamycin (40%), pristinamycin (20%), fusidic acid (40%), and rifampicin (60%). In the Republic of Congo, a study conducted by [12] in *S. aureus* isolated at the Brazzaville University Hospital Centre (CHUB) showed resistance rates for penicillin G 55.10%, oxacillin 53.06%, kanamycin 14.26%, tobramycin 12.24%, and gentamycin 42.55%. These results differ from those reported in this study. This difference could be explained by the fact that acquired resistance is evolutionary, it evolves with time and location. [13] in Morocco, reported for vancomycin, gentamicin, ciprofloxacin, erythromycin, pristinamycin and fusidic acid whose respective resistance rates are: 0%; 66.6%; 72.2%; 50%; 16.6% and 61.1%.

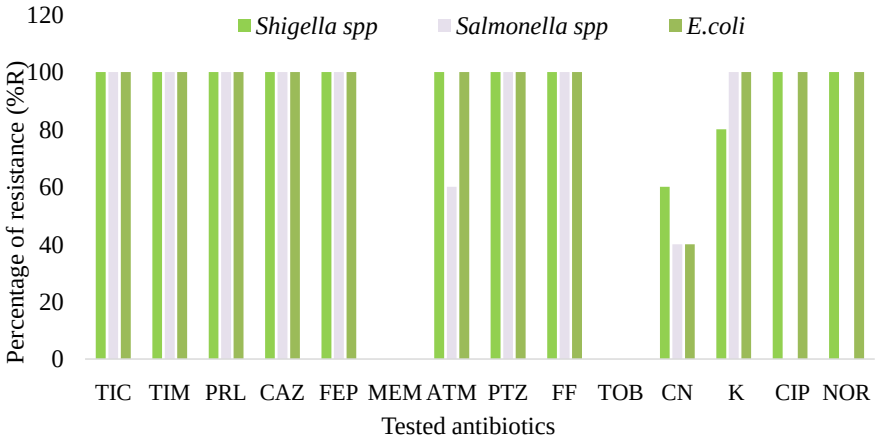

Figure 4. Antibiotic resistance profile of *Shigella* spp, *Salmonella* spp and *E. coli*.

These rates are quite close to those reported we found. A study conducted by [14] in Congo Brazzaville on community and clinical settings showed similar rates on community strains to those found in this study. Beta-lactam resistance was dominated by the POXACX phenotype, with similar results reported by [15]. This could be due to the *mecA* gene encoding a PLP2a. Resistance to MLSB was marked by the EDAPT phenotype. This could be due to *ErmC*-type methylases.

Bacillus cereus is ubiquitous in nature and is easily spread through food production systems. Contamination by this species is almost inevitable. *B. cereus*, was reported to be resistant to penicillin G and sensitive to aminoglycosides, fluoroquinolones, glycopeptides, lincosamides and rifampicin [16]. These are explained by the fact that *B. cereus* produce beta-lactamases and are therefore considered resistant to beta-lactam antimicrobial agents [17]. In our study, the resistance rates for penicillin G, oxacillin, erythromycin, gentamycin, clindamycin and rifampicin were 80%, 100%, 40%, 20%, 80% and 80% respectively. These results differ from those reported by [16] in Egypt on the prevalence of *Bacillus cereus* resistance, where the respective rates were 100% for penicillin G and 0% for the rest of the above antibiotics. The work carried out by [18] in Ghana on the prevalence of virulence factors and antibiotic resistance genes in *Bacillus cereus* shows resistance rates of 88% for oxacillin and 0% for the same antibiotics mentioned previously.

All *E. coli* strain isolates were resistant to Cefrazidime. This differs from rate of resistant of 77.3% reported by [19] on *E. coli* isolated at the CHUB. [20] in the Republic of Congo working on *E. coli* isolated from CHUB inpatients and outpatients reported resistance rates of 46.51%, 37.20%, 34.88% 13.95% and 46.51% respectively for piperacillin-tazobactam, cefepime, gentamicin, ciprofloxacin and fosfomycin in the extended-spectrum beta-lactamase producing strains. These levels differ from those reported in our study. [13] in Morocco reported resistance rates of 60.2% for gentamycin, 75.5% for ciprofloxacin and 75.5% for norfloxacin. These differ from those we found for norfloxacin, ciprofloxacin and

gentamycin in *E. coli* where the resistant rates were 100%, 100% and 40% respectively.

The genus *Salmonella* is one of the leading causes of collective foodborne illness. Although some cases may be directly transmitted from pets, reptiles or contaminated water, the percentage of transmission through food is estimated at 95% [21]. The results of our study show that these strains presented resistance rates of 100% to kanamycin, piperacillin-tazobactam, cefepime and 0% to ciprofloxacin and tobramycin. In Chad, [22] reported resistance rates of 10.5%; 0%; 11%; 0%; 31.8% for kanamycin, piperacillin-tazobactam, cefepime, ciprofloxacin and tobramycin respectively in *Salmonella* strains isolated from semi-traditional farmers. On the other hand, traditional farmers reported resistance rates of 14.29%; 0%; 0%; 0% and 19.05% respectively for the same antibiotics.

Antibiotic sensitivity testing showed that *Shigella* spp isolates were 100% resistance to ciprofloxacin and ceftazidime. In a study conducted by [23] in America, resistance rates of 50% to ciprofloxacin and 0% to ceftazidime were reported. The work of [24] [25] revealed a total sensitivity of the strains to ciprofloxacin and gentamycin. Our results on kanamycin (80%) differed from those found by [26] (0%). In Asia, 81.8% resistance to norfloxacin was demonstrated [27] while it was 100% in our results. With piperacillin, our results are consistent with those of [28], where 100% resistance was observed. The presence of the TIC TIM PRL TPZ CAZ FEP ATM beta-lactam resistance phenotype in *E. coli*, *Salmonella* spp and *Shigella* spp could be explained by inactivation of these antibiotics by extended-spectrum beta-lactamases.

The presence of these pathogens in smothered fish could be explained by human activity (hands, kitchen utensils, water, ingredients, packaging sheets) on the one hand and by the microbial flora of the fish on the other hand. The resistance of the bacteria to the cooking temperature would be explained either by sporulation as is the case for *Bacillus cereus* or by the short cooking time. The high resistance rates observed could be explained by the dissemination of resistance genes between bacteria by horizontal and vertical transfers.

5. Conclusion

The study showed that the pathogenic bacteria *S. aureus* and *B. cereus* contained in the smothered fish are highly resistant to beta-lactam antibiotics and MLSB, while the enterobacteria *Shigella* spp and *E. coli* were resistant to beta-lactam and quinolones. Beta-lactam and MLSB resistance in Gram-positive bacteria have been characterized by the presence of resistance phenotypes highlighting the involvement of penicillinase (PLP2a) and methylasses (*ErmC*) respectively. In contrast, in Gram-negative bacteria, the resistance phenotype reporting the production of extended-spectrum beta-lactamases was observed to beta-lactam. However, vancomycin, kanamycin, tobramycin, gentamycin, ciprofloxacin, norfloxacin can be used for the treatment of community-acquired infections caused

by Gram-positive bacteria; meropenem and tobramycin for infections of *Shigella* spp, *Salmonella* spp and *E. coli*.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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