



Antibiotic sensitivity of bacteria isolated from the oral cavities of live white sharks (*Carcharodon carcharias*) in South African waters

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The white shark (*Carcharodon carcharias*) is responsible for 49% of shark-related injuries in South Africa, yet no information currently exists on the composition or antibiotic resistance of bacteria hosted by these apex predators in South African waters. This study aimed to address this gap by sampling the bacteria present in the oral cavities of 28 live *C. carcharias* along South Africa's southern coastline. The antibiotic resistance of the range of microbiota was also assessed using antibiotic disc diffusion tests. A total of 51 strains from at least 20 species of bacteria were isolated from the oral cavities of *C. carcharias*. Of these strains, the most common bacteria present were *Serratia* spp., *Proteus vulgaris* and *Vibrio alginolyticus*. The overall antibiotic resistance was relatively higher in this study than that reported for bacterial microbiota sampled from other shark species. Results indicate that the combination therapy of imipenem (carbapenem antibiotic) and vancomycin (glycopeptide antibiotic) might be the most parsimonious option to effectively treat infections resulting from white shark bites, particularly in South Africa. It is hoped that, in addition to assisting medical professionals to treat shark bite victims, these findings enhance the understanding of the microbial communities present in large coastal predators and their surrounding environments.

Significance:

- Overall antibiotic resistance of bacteria in the oral cavities of *C. carcharias* was relatively high.
- Combination therapy of imipenem (carbapenem antibiotic) and vancomycin (glycopeptide antibiotic) is recommended for the treatment of white shark bites, particularly in South Africa.
- The findings add to understanding of the microbial communities present in large coastal predators and their surrounding environments.

Introduction

The oral cavity of sharks, like many fauna, is host to a wide range of bacteria.¹⁻⁴ Therefore, victims of shark-related injuries involving shark bites require treatment for the prevention of infections caused by the transfer of pathogenic bacteria.^{5,6} A review of 11 recent shark-related injuries in the USA indicated that only three of the reviewed patients received an appropriate selection of antibiotics for treatment of infection (using ciprofloxacin), and none of the reviewed patients received dual antibiotic therapy.⁵ An earlier review of 83 shark-related injuries in South African waters could only confirm that 18 of the reviewed patients received any antibiotic treatment (using a variety of different antibiotics), and three of these patients continued to develop septic complications that required further surgical intervention.⁶ Currently, there is no consensus on the most appropriate antibiotics to be used in treating bacterial infections resulting from shark-related injuries due to large differences in the composition of bacterial microbiota present among different shark species and their geographical locations.^{4,5} Therefore, further information is needed on the antibiotic resistance of bacteria present in the oral cavities of different shark species from different regions of the world that might be responsible for shark-related injuries in humans.

There have been increasing reports of antibiotic resistance among bacteria hosted by marine predators^{3,4,7}, possibly because of the increased use of broad-spectrum antibiotics in humans and their subsequent entry as contaminants into coastal waters⁷. A study of bacteria found post-mortem in the oral cavities of bull sharks (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*) off Recife (Brazil) reported high levels of antibiotic resistance among several of the 81 isolated bacterial strains.³ In particular, that study found a 20% resistance among *Proteus mirabilis* strains to imipenem, a broad-spectrum antibiotic commonly used to treat Gram-negative nosocomial infections.⁸ Another study on bacteria isolated from cloacal swabs of *C. leucas*, blacktip sharks (*Carcharhinus limbatus*), nurse sharks (*Ginglymostoma cirratum*) and lemon sharks (*Negaprion brevirostris*) from Belize and the US East Coast, reported multidrug resistance in bacteria tested from all sampled shark species.⁷ A comprehensive study undertaken by Unger et al.⁴ of bacteria isolated from the oral cavities of adult, live *C. limbatus* in Florida (USA) found that the three primary bacterial types present were *Vibrio* spp., *Staphylococcus* spp. and *Pasteurella* spp. Unger et al.⁴ additionally reported that 43% of isolated bacteria showed resistance to at least one antibiotic, and the overall resistance rate of all antibiotics they tested was 12%. Despite the prevalence of antibiotic resistance, these authors concluded that the best antibiotic selection for treating infections resulting from shark-related injuries involving *C. limbatus* near Florida was either a broad-spectrum fluoroquinolone, or a dual antibiotic treatment using a third-generation cephalosporin and doxycycline.⁴

South Africa has the third highest incidence (after the USA and Australia) of human/shark encounters from 1580 to the present.⁹ Within South Africa, white sharks (*Carcharodon carcharias*) account for 49% of reported shark-related injuries.⁶ However, data on the bacterial microbiota present in *C. carcharias* oral cavities are limited to one sample that was taken post-mortem from a harpooned shark in northeast USA over two decades ago.² At present, very little is known about the full composition of bacterial strains present in *C. carcharias* oral cavities, or what the current resistance of these bacteria might be to available antibiotics. This study aimed to address this informational gap in order to assist medical professionals in making informed decisions when administering treatment for bacterial

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infections in *C. carcharias* bite victims. Specifically, this study aimed to identify bacterial species that are present in the oral cavities of live *C. carcharias* along the southern coastline of South Africa, and to assess the sensitivity of these bacteria to 16 commonly used antibiotics. It is intended that these results might help in selecting the most appropriate antibiotic(s) for this category of injury, and also enable alternatives to broad-spectrum antibiotics that continue to raise resistance levels of pathogenic microbiota.

Methods

Animal capture and sample preparation

A total of 28 *C. carcharias* were caught using circle hooks tethered to floating buoys from four sample sites along South Africa's southern coastline (False Bay, Gansbaai, Mossel Bay and Algoa Bay) and sampled for this study (Figure 1). Sharks were led to a submerged platform, which was then lifted out of the water to minimise cross-contamination while sampling. Water supply to the gills was achieved via pumping seawater through a rigid pipe inserted into the mouth of the shark. Sterile cotton swabs were used to collect microbial samples from sharks' oral cavities. To ensure comprehensive sampling, separate swabs were taken of sharks' teeth, gums and tongue. However, these data were compiled together into a single sample category (oral cavity) due to likely cross-contamination. Additionally, attention was given to avoiding contact with the rigid pipe while sampling in order to minimise the chance of cross-contamination among individuals. All sharks were handled for a maximum of 15 min, after which they were released and monitored by a veterinarian to ensure no external signs of stress and/or capture myopathy. All interactions were approved by the South African Department of Environmental Affairs (reference RES2012/OCEARCH/JOHNSON).



Figure 1: Capture locations for white sharks (*Carcharodon carcharias*) sampled in this study.

Processing and identification of bacteria

All swabs were transported to the Ampath pathology laboratory in George (Western Cape, South Africa) within a week of sampling, where bacterial samples were each plated onto three replicates of Nutrient Agar[®] and streaked for single colonies. They were then incubated for 24 h at 37 °C in an aerobic atmosphere and stored in Tryptone Soya Broth[®]. Presumptive isolates from the Nutrient Agar[®] replicates were recovered from all plates, sub-cultured for purity and stored in Tryptone Soya Broth[®] plus 10% glycerol within microbank cryovial systems at -20 °C until further processing. Initial screening of presumptive isolates began with examining for Gram status, lactose fermentation, as well as oxidase and catalase reactions. Identifications of Gram-negative bacteria were confirmed using bioMérieux[®] API[®] 20E systems. Where possible, Gram-negative isolates were identified to species level, but in some cases could only be identified to genus level. Inconclusive Gram-negative isolates were reported as 'Gram-negative bacilli'. Gram-positive isolates were identified using Biorad Pastorex Staph Plus[®] and Remel[®] Streptex[®] kits.

Determining presence of beta-lactamase production

All confirmed bacterial strains were additionally examined for chromosomal beta-lactamase (Amp-C) production and extended beta-lactamase (ESBL) production at the Ampath pathology laboratory, as bacterial strains that can produce either type of these enzymes are likely to have strong resistance to all beta-lactam type antibiotics.¹⁰ Amp-C production was assessed by observing a flattening of the cefotaxime zone on the side of the microbe adjacent to imipenem, as well as a flattening of the ceftazidime and/or cefotaxime zone on the side of the microbe adjacent to ceftazidime. Determining the presence of ESBL production among bacterial strains was performed by observing distortions of the inhibition zones around ceftazidime and/or cefepime, in the areas adjacent to the amoxicillin/clavulanate disc. Distortions indicating ESBL production took the form of (1) an increased radius of the inhibition zone for the cephalosporin(s) or (2) the presence of a lens-shaped inhibition zone between the cephalosporin and amoxicillin-clavulanic discs either when there was no inhibition zone around the cephalosporin disc or if the inhibition zone was very narrow.

For bacterial strains in which Amp-C production was present, resistance to ampicillin; amoxicillin/clavulanic acid; piperacillin/tazobactam; first-, second- and third-generation cephalosporins; and cefoxitin was reported, regardless of zone size or further testing. For any bacterial strains for which ESBL production was confirmed, resistance to ampicillin, first-, second-, third- and fourth-generation cephalosporins, piperacillin/tazobactam, amoxicillin/clavulanic acid, as well as sensitivity to cefoxitin was reported. The susceptibilities of these strains to other antibiotics, not listed above, were reported according to results of the further testing outlined below.

Assessing bacterial sensitivity to antibiotics

All bacterial strains isolated from oral cavity samples of *C. carcharias* were tested for susceptibility to a panel of 16 antibiotics (summarised in Table 1) using antibiotic disc diffusion tests according to the Kirby–Bauer methodology outlined in the guidelines of the US Clinical and Laboratory Standards Institute.¹¹

Table 1: Classes and types of antibiotics used in disc diffusion tests to assess the antibiotic sensitivity of bacterial flora isolated from *Carcharodon carcharias* oral cavities

Antibiotic used in disc diffusion test	Abbreviation	Dosage	Class / type of antibiotic
Amikacin	AMK	30 mcg	Aminoglycoside
Imipenem	IPM	10 mcg	Carbapenem
Cefepime / cefpirome	FEP	30 mcg	Cephalosporin
Ceftazidime	CAZ	30 mcg	Cephalosporin
Ceftriaxone / cefotaxime	CTR	30 mcg	Cephalosporin
Cefuroxime	CFX	30 mcg	Cephalosporin
Cephalothin	CEP	30 mcg	Cephalosporin
Ciprofloxacin	CIP	5 mcg	Fluoroquinolone
Trimethoprim / sulfamethoxazole	SXT	25 mcg	Folate pathway inhibitor
Vancomycin	VAN	30 mcg	Glycopeptide
Amoxicillin	AMOX	30 mcg	Penicillin
Amoxicillin / clavulanic acid	AMC	30 mcg	Penicillin
Penicillin	PEN	10 mcg	Penicillin
Piperacillin / tazobactam	PTZ	110 mcg	Penicillin
Erythromycin / clarithromycin / azithromycin	ERY	15 mcg	Macrolide
Tetracycline	TET	30 mcg	Tetracycline

Bacteria were reported as sensitive to an antibiotic if all strains of those bacteria indicated sensitivity to the drug. Bacteria were reported as resistant to an antibiotic if all of its strains were resistant to the drug. If different strains of the same type of bacteria showed conflicting susceptibility to an antibiotic it was reported in the results as 'R*', but was considered resistant for the sake of calculating relative percentages of antibiotic effectiveness. If an antibiotic is not routinely used on an isolate, is not used for treatment of a given strain, or if there was no available information on the zone or the minimum inhibitory concentration breakpoint of the bacteria or antibiotic¹¹, then this combination was reported in the results as 'not applicable'.

Results

Bacterial species present

A total of 51 bacterial strains were isolated from oral cavities of 28 live *C. carcharias* (Table 2). Among these strains, there were at least 20 species of bacteria present. An additional five strains which could not be identified were grouped into the category 'Gram-negative bacilli' (Table 2). The three most common bacterial strains identified from the oral cavities (in order of frequency) were: *Serratia* spp., *Proteus vulgaris* and *Vibrio alginolyticus*. *Aeromonas hydrophyla*, *Enterococcus faecalis*

and *Staphylococcus* spp. were also present in at least 10% of all sharks sampled (Table 2).

Assessment of Amp-C and ESBL production among isolated strains

Among the 51 bacterial strains isolated from samples, 6 isolates tested positive for Amp-C production. These isolates were: *E. cloacae* ($n=2$), *Klebsiella pneumoniae* ($n=1$), *Proteus mirabilis* ($n=1$), *P. vulgaris* ($n=1$) and *Serratia* spp. ($n=1$). All strains tested negative for ESBL production.

Bacterial sensitivity to antibiotics

All strains of bacteria isolated from the oral cavities of live *C. carcharias* in this study displayed sensitivity to a panel of at least four types of antibiotics (Table 3). Antibiotic resistance varied greatly among bacterial strains, but was observably higher in *E. cloacae*, *K. pneumoniae*, *P. mirabilis*, *P. vulgaris* and *Serratia* spp. which were among the strains that tested positive for Amp-C production. Of the 16 antibiotics that were tested, ciprofloxacin, amikacin and imipenem yielded the highest sensitivity among bacterial strains (Table 3). Specifically, these three antibiotics were effective in treating each of the Amp-C positive bacterial strains mentioned above. Trimethoprim-sulfamethoxazole and tetracycline followed closely in overall effectiveness; however, several of the Amp-C positive bacteria were resistant to both these antibiotics (Table 3). Isolated bacterial strains showed the highest resistance to amoxicillin, piperacillin-tazobactam and the cephalosporins (cephalothin and cefuroxime). No single antibiotic tested could effectively treat all the bacterial strains on its own (Table 3), indicating that multiple antibiotics would be necessary in treating exposure to the full panel of bacteria isolated from *C. carcharias* oral cavities in this study.

Discussion

Among the 16 antibiotics tested, both imipenem and ciprofloxacin were the only two that effectively treated each of the Gram-negative bacteria found in the oral cavities of *C. carcharias* in this study (Table 3). The Gram-positive bacteria isolated from *C. carcharias* oral cavities included *Enterococcus* spp., *Staphylococcus* spp. and *Streptococcus* spp. and these strains showed consistent sensitivity to both vancomycin and penicillin during disc diffusion tests (Table 3). Infection with these Gram-positive bacteria may also be effectively treated with imipenem; however, at the time of writing, there was no available information on the zone or minimum inhibitory concentration breakpoint of enterococci, staphylococci or streptococci with imipenem¹¹, and so this combination was not assessed in the disc diffusion test in the present study. Therefore, these findings indicate that treatment with imipenem, and possibly a combined regime of vancomycin would be the most effective option for preventing and/or treating infections resulting from white shark bites in South Africa. Based on the results, ciprofloxacin and penicillin, in place of imipenem and vancomycin respectively, also have potential as treatment options for white shark bite patients (Table 3). However, fluoroquinolone antibiotics, to which class ciprofloxacin belongs, often show an antagonistic effect in antibiotic combination therapies, as can the doubling up of two beta-lactam antibiotics¹² (e.g imipenem and penicillin). Nevertheless, Al-Hasan et al.¹³ demonstrated that fluoroquinolones combined with beta-lactam antibiotics can contribute to a positive treatment outcome when suspecting Gram-negative bacilli. That said, combination therapy between fluoroquinolones and beta-lactam antibiotics should be considered carefully as the synergistic significance is not clear and clinical outcomes are conflicting.¹⁴

The overall composition of bacterial microbiota identified in the oral cavities of live *C. carcharias* in this study was similar to microbial presence reported from other shark species.^{3,4} Specifically, the prevalence of *Enterobacter* spp. and *Proteus* spp. coincided with common bacteria sampled from *C. leucas* and *G. cuvier* in Recife, Brazil.³ The occurrence of *Vibrio* spp. and *Staphylococcus* spp. strains mirrored findings from live *C. limbatus* oral cavities in Florida, USA.⁴ Additionally, the presence of *Vibrio* spp. and *Shewanella putrefaciens* was consistent with bacterial microbiota previously reported from the teeth of *C. carcharias*.² However, findings from the present study also indicate a high incidence of many other bacterial microbiota, specifically *Enterococcus* spp. and *Serratia* spp. in



the oral cavities of *C. carcharias* in South African waters. It is additionally worth noting that the delay of up to 1 week for transporting some of the swab samples to the laboratory may have biased our findings towards the presence of faster-growing and/or persistent bacterial species. Nonetheless, at least 20 distinct species of bacteria were identified from the oral cavities of live sharks in this study, all of which could pose risk of pathogenesis to patients with shark-related injuries.

The overall antibiotic resistance observed in this study was relatively higher than that reported in other shark species.^{3,4,7} Specifically, no single antibiotic tested in this study would be capable of effectively treating all pathogenic bacteria presently reported from *C. carcharias* oral cavities. This is in contrast to studies of other shark species, which have suggested that a single agent fluoroquinolone (ciprofloxacin or levofloxacin) could be used for treatment of infections in *C. leucas*, *C. limbatus* and *G. cuvier* bite victims.^{3,4} Buck et al.² who assessed the antibiotic resistance of four bacterial strains isolated from the teeth of one *C. carcharias* sampled from northeast USA, reported that effective treatment of these bacteria could be achieved using aminoglycosides, tetracycline or several cephalosporin class antibiotics. These differences

in bacterial composition and resistance levels reflect the dynamic nature of bacteria over space, time and host species^{7,15}, as well as highlight the need for species and geographic-specific microbial assessments.

Due to the limited sample size and sampling region, comparison of *C. carcharias* bacterial compositions related to geography, host age, maturity and diet were outside the scope of this study. Nonetheless, these factors are likely to have strong impacts on the presence and antibiotic resistance of pathogenic bacteria affecting shark-related injury victims.^{4,7} Preliminary studies have indicated no discernible relationship between the composition of oral bacteria in *C. carcharias* and either their gut contents or sampling location in South Africa.¹⁶ These patterns suggest that differences in feeding ecology and fine-scale geography might have minimal impact on the bacterial compositions hosted by these highly migratory¹⁷ apex predators in South African waters. However, it is highly advocated that further microbial studies addressing the sharks' sex, age and maturity stage, as well as movement patterns over a global scale, would be very useful for making inferences between the life history of this species and the microbiota that it hosts.

Table 2: Bacterial species identified from swab samples taken from the oral cavities of 28 live white sharks (*Carcharodon carcharias*)

	False Bay			Gansbaai						Struisbaai			Mossel Bay										Algoa Bay	%						
Shark	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		24	25	26	27	28	
Sex	F	F	F	M	M	F	M	F	M	M	F	F	M	F	F	F	F	F	F	F	F	F	F		F	M	F	M	F	
Total length (cm)	417	437	431	318	297	360	355	452	330	251	505	441	382	340	299	351	283	271	228	350	297	292	251		259	266	340	309	265	
<i>Aeromonas hydrophyla</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	P	-	-	P	-	-	10.71	
<i>Eikenella corrodens</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	P	-	-	-	7.14	
<i>Enterobacter amnigenus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	3.57	
<i>Enterobacter cloacae</i>	-	-	-	P	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.14	
<i>Enterococcus faecalis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	P	P	-	10.71
<i>Enterococcus faecium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	3.57	
<i>Escherichia coli</i>	-	-	-	-	-	-	-	-	-	-	P	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.14
<i>Klebsiella pneumoniae</i>	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.57
<i>Pantoea</i> spp.	-	-	P	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.14
<i>Photobacterium damsela</i>	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.57
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	P	-	-	-	7.14
<i>Proteus vulgaris</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	P	-	-	P	-	-	-	P	P	17.86
<i>Pseudomonas oryzae</i>	-	-	-	-	-	-	-	-	-	-	P	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.14
<i>Pseudomonas fluorescens</i>	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	7.14
<i>Serratia</i> spp.	P	P	P	-	-	-	P	-	P	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21.43
<i>Shewanella putrefaciens</i>	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	7.14
<i>Staphylococcus</i> spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	P	-	-	-	-	-	-	P	-	-	-	-	-	10.71
<i>Streptococcus</i> spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.57
<i>Vibrio alginolyticus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	P	-	P	-	-	-	-	-	P	-	-	14.29
<i>Vibrio parahaemolyticus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	3.57
Gram-negative bacilli	P	-	-	-	P	-	-	P	-	-	-	-	P	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	17.86

P, present. The last column represents the raw percentage of sampled sharks that were hosts to the specified bacterial strain.

Table 3: The 20 bacterial strains and Gram-negative bacilli isolated from oral cavity samples of *Carcharodon carcharias* and their corresponding sensitivity to 16 common antibiotic types

Bacteria	Antibiotics tested															
	AMK	IPM	FEP	CAZ	CTR	CFX	CEP	CIP	SXT	VAN	AMOX	AMC	PEN	PTZ	ERY	TET
<i>Aeromonas hydrophyla</i>	S	S	S	S	S	–	–	S	S	–	–	S	–	–	–	S
<i>Eikenella corrodens</i>	–	S	–	–	S	–	–	S	S	–	–	S	–	–	–	S
<i>Enterobacter amnigenus</i>	S	S	S	S	S	–	–	S	S	–	–	S	–	–	–	S
<i>Enterobacter cloacae</i>	S	S	R*	R*	R*	R	R	S	R*	–	R	R*	–	R	–	S
<i>Enterococcus faecalis</i>	–	–	–	–	–	–	–	–	–	S	–	–	S	–	–	–
<i>Enterococcus faecium</i>	–	–	–	–	–	–	–	–	–	S	–	–	S	–	–	–
<i>Escherichia coli</i>	S	S	S	S	S	–	–	S	S	–	–	S	–	–	–	S
<i>Klebsiella pneumoniae</i>	S	S	S	R	R	R	R	S	S	–	R	R	–	R	–	S
<i>Pantoea</i> spp.	S	S	R*	R*	R*	–	–	S	S	–	–	R	–	–	–	S
<i>Photobacterium damsela</i>	S	S	S	S	S	–	–	S	S	–	–	S	–	–	–	S
<i>Proteus mirabilis</i>	S	S	S	R*	R*	R	R	S	S	–	R	R*	–	R	–	R
<i>Proteus vulgaris</i>	S	S	R*	R*	R*	R	R	S	S	–	R	R*	–	R	–	R*
<i>Pseudomonas oryzihabitans</i>	S	S	–	S	–	–	–	S	–	–	–	–	–	–	–	–
<i>Pseudomonas fluorescens</i>	S	S	S	S	–	–	–	S	S	–	–	–	–	–	–	–
<i>Serratia</i> spp.	S	S	S	R*	R*	R	R	S	R*	–	R	R*	–	R	–	S
<i>Shewanella putrefaciens</i>	S	S	–	S	S	–	–	S	S	–	–	R*	–	–	–	S
<i>Staphylococcus</i> spp.	S	–	–	–	–	–	S	S	S	S	S	S	S	–	S	S
<i>Streptococcus</i> spp.	–	–	–	–	S	–	–	–	–	S	S	–	S	–	S	S
<i>Vibrio alginolyticus</i>	S	S	S	S	S	–	–	S	S	–	–	S	–	–	–	S
<i>Vibrio parahaemolyticus</i>	S	S	S	S	S	–	–	S	S	–	–	S	–	–	–	S
Gram-negative bacilli	S	S	S	S	S	–	–	S	S	–	–	R*	–	–	–	S
Antibiotic effectiveness (%)	80.95	80.95	52.38	47.62	47.62	0	4.76	85.71	71.43	19.05	9.52	38.10	19.05	0	9.52	71.43

S, sensitive; R, resistant; R*, at least one strain of this bacteria was resistant to the corresponding antibiotic; –, test not applicable to isolate; AMK, amikacin; IPM, imipenem; FEP, cefepime/cefpirome; CAZ, ceftazidime; CTR, ceftriaxone/cefotaxime; CFX, cefuroxime; CEP, cephalothin; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; VAN, vancomycin; AMOX, amoxicillin; AMC, amoxicillin/clavulanic acid; PEN, penicillin; PTZ, piperacillin/tazobactam; ERY, erythromycin/clarithromycin/azithromycin; TET, tetracycline

Conclusion

This study presented the first assessment of antibiotic resistance among bacterial strains isolated from the oral cavities of live *C. carcharias* in South Africa. Bacteria identified in this study include pathogenic bacteria previously unreported for shark species occurring in South Africa. It is hoped that the data presented here can enable medical professionals to make more informed, and thus more effective decisions when administering antibiotic treatment to shark bite victims, and provide an increased understanding of the microbial communities present in large coastal predators and their surrounding environments. The antibiotic resistance reported here of bacterial microbiota hosted by these top predators will additionally serve as baseline information toward future studies and management processes serving human public health.

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Authors' contributions

This study was conceived and designed by E.G. Data were collected by E.G., A.A.K., M.J.S., A.T., C.F., R.J. and M.M. Data were validated by

L.A.B. and curated by P.M., N.K., L.A.B. and E.G. An initial draft of this manuscript was produced by E.G., N.K. and L.A.B. Funding was acquired by C.F. and R.J. This project was overseen and managed by E.G. P.M. interpreted the data and was primary author of the final manuscript. All authors contributed to revisions of the final draft of the manuscript.

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