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Research Article

Incidence of multi drug resistant coagulase-negative Staphylococci from seafood samples, Veraval, Gujarat

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Abstract

In earlier days, Coagulase-negative *Staphylococcus* (CoNS) were considered as a non-pathogenic or opportunistic pathogen, and much attention was not given to them. Recently, due to the transfer of resistant genes from non-pathogen to opportunistic pathogens leads to a severe illness in human clinical cases. In most of the food materials, CoNS with multi-drug resistance (MDR) were reported. But, very scant information is available on the different proportions of the CoNS and its resistance pattern to different classes of antibiotics. In the present study, a total of 31 CoNS consists of six different species like *S. warneri* (12), *S. haemolyticus* (6), *S. hominis* (4), *S. saprophyticus* (3), *S. simulans* (3) and *S. xylosus* (3) were isolated from 31 seafood samples including fin fishes, shrimp, lobster and octopus, and identified using API biomerieux strips. The antibiogram results of high resistance rates against gentamicin (70.90%), azithromycin (64.50%) and tigecycline (32.20%) were detected. Thus, the findings suggest that CoNS in seafood samples can have a potential role as a reservoir for antimicrobial resistance and its transfer.

Key words: Antimicrobial resistance, Coagulase-negative *Staphylococcus* (CoNS), Opportunistic infections

Highlights

- Thirty one CoNS strains were isolated from 31 retail seafood samples of Veraval, Gujarat
- Higher level of antimicrobial resistance to gentamicin, azithromycin and tigecycline was noted.
- All the isolates showed multi-drug resistance with resistance to more than three antibiotic classes.
- The consumption of raw seafood is likely to transmit multidrug-resistant Staphylococci to humans.

INTRODUCTION

The knowledge about the non-pathogenicity of coagulase-negative Staphylococci (CoNS) has been challenged during the last few decades with inventions like hospital-acquired infections and its multiple drug resistance (MDR). Among them, *Staphylococcus epidermidis* and *S. haemolyticus* are the most significant species of nosocomial infections. Several reports are available for the potential pathogenicity (Gillespie *et al*., 2009) and presence of resistance genes (Corrente *et al*., 2009; Podkowik *et al*., 2012) in various CoNS. But information on the spread of antimicrobial resistance in foodborne pathogens is largely restricted to developed countries. The CoNS have also been causing toxic shock syndrome (TSS) and Staphylococcal food poisoning (SFP), as they carry enterotoxin genes (Becker *et al*., 2014). Thus, ready to eat food products having drug-resistant CoNS may lead to a potential health risk because of their ineffectiveness to drugs and ability to transfer resistance genes to humans (Walther and Perreten, 2007). Several studies have been conducted to determine the antimicrobial resistance profile of CoNS from different food items. Batista *et al*. (2013) identified CoNS in

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marine clam *Anomalocardia brasiliana* from the harvesting area and retail shops. Sampimon *et al*. (2011) examined the resistance of CoNS from bovine milk against penicillin, oxacillin and macrolide–lincosamide (ML) antibiotics. Chajecka-Wierzchowska *et al*. (2014) checked the antimicrobial resistance profile of CoNS isolated from ready-to-eat food of animal origin like cheeses, cured meats, sausages, smoked fishes etc. and found resistance to cefoxitin, clindamycin, tigecycline, rifampicin and erythromycin. Likewise, Regecova *et al*. (2014) found some antimicrobial resistance of CoNS isolated from the marine fish. In a similar way, clams were found to contain novobiocin resistant *S. cohnii* subsp. *Urealyticus*, *S. lentus, S. sciuri* subsp. *Sciuri*, and *S. xylosus* (Becker *et al*., 2014). Hammad *et al*. (2012) isolated methicillin-resistant CoNS from Japanese retail ready-to-eat raw fish (Sashimi) for the first time. Most Indian consumers prefer the fish in the retail fish market. But, these retail fish markets are an important source of the spread of MDR bacteria (Visnuvinayagam *et al*., 2015) due to the repeated use of the same water for washing the fish (Visnuvinayagam *et al*., 2016, 2017). Even though a considerable number of reports are available on CoNS, scant reports on the different species existed in the particular locality with detailed resistance analysis. Thus, the study aimed to identify the presence of CoNS in various seafood samples from Veraval, Gujarat and its phenotypic antimicrobial resistance, with emphasis on clinically relevant antibiotics.

MATERIALS AND METHODS

Sample collection and identification of Staphylococci: Thirty-one seafood samples comprising fish (23), shellfishes [Shrimp (1), lobster (2)], and molluscs [octopus (5)] were collected from the Veraval region, Gujarat, and immediately transported to the laboratory in portable coolers and analyzed within 2 hours.

Isolation of CoNS was carried out as per ISO: 6888-1. About 25 g of product, mixed with 225 mL of normal saline (0.85%) with 0.1% peptone was homogenized in a stomacher masticator (Seward, UK). Aliquots of each serially diluted homogenate were spread onto Baird Parker agar (Oxoid, England) supplemented with egg yolk tellurite emulsion. Plates were incubated at 35°C±2°C for 48 h. According to the manufacturer's instructions, three typical colonies were chosen to identify CoNS by the API-Staph Biomerieux identification system (APIS). After the identification, different species were selected for the antibiotic sensitivity assay (ASA). If one sample consisted of the same species, one was selected. Totally 31 strains of Staphylococci were identified from 31 seafood samples through APIS.

Confirmation of coagulase activity: As per Sperber and Tatini (1975), individual colonies were reinoculated into 2 mL of Brain Heart Infusion Broth (Oxoid, England) and incubated for 18 to 24 h at 37°C. A volume of 0.2 mL of this overnight culture was added to 0.5 mL of rabbit plasma (with EDTA) (BD BBL™) in a tube, then incubated at 35°C. Formation of coagulum/ clot was considered as a positive reaction. The confirmed coagulase-negative isolates were stored at -20°C for further studies.

Phenotypic antimicrobial resistance: The identified strains were assessed for antimicrobial sensitivity against 24 antimicrobial agents using Dodeca Staphylococci 1 and 2 (HiMedia, Mumbai, India) by disc diffusion method (Baur *et al*., 1966) on Mueller Hinton agar with 4% NaCl. The antibiotics tested comprised aminoglycosides (gentamicin), beta-lactam and combinations (cefoxitin, ampicillin/sulbactam, piperacillin/tazobactam), quinolones (ciprofloxacin, gatifloxacin, ofloxacin, lomefloxacin, moxifloxacin and norfloxacin), glycopeptides (teicoplanin and vancomycin), lincosamides (clindamycin), macrolides (erythromycin, azithromycin, and clarithromycin), penicillins (penicillin), streptogramins (pristinamycin), aminocoumarin (novobiocin), rifamycin (rifampicin), glycylcyclines (tigecycline), nitrofurantoin, oxazolidinones (linezolid) and sulphonamides (co-trimoxazole). The results were interpreted as per CLSI guidelines (2015).

RESULTS

The present study evaluated the prevalence of CoNS in 31 retail seafood samples of Veraval, Gujarat, and a total of 31 strains were identified. The dominant species were *S. warneri* (12), *S. haemolyticus* (6), *S. hominis* (4), *S. saprophyticus* (3), *S. simulans* (3) and *S. xylosus* (3). The distribution of different isolates per sample is shown in Table 1. All the isolates were identified as CoNS spp. by the

Table 1. Number of coagulase-negative Staphylococci isolates from different seafood samples

CoNS spp.	Fish (n= 23)	Octopus (n= 5)	Lobster (n= 2)	Shrimp (n=1)	Total (n= 31)
S. warneri	10	2	-	-	12
S. haemolyticus	4	1	-	1	6
S. hominis	3	-	1	-	4
S. saprophyticus	2	1	-	-	3
S. simulans	2	-	1	-	3
S. xylosus	2	1	-	-	3

Table 2. Phenotypic resistance profile including number of resistant (R), intermediately susceptible (I), and susceptible (S) isolates of coagulase-negative Staphylococci from different seafood samples

Anibiotics	Fish (n= 23)			Octopus (n= 5)			Lobster (n= 2)			Shrimp (n=1)		
	R	I	S	R	I	S	R	I	S	R	I	S
Penicillin-G (10 unit)	3	-	20	3	-	2	1	-	1	-	-	1
Erythromycin (15 µg)	2	4	17	-	1	4	1	1	-	-	1	-
Clarythromycin (15 µg)	2	-	21	-	-	5	1	-	1	-	1	-
Azithromycin (15 µg)	14	-	9	5	-	0	1	-	1	-	-	1
Linezolid (30 µg)	1	-	22	-	-	5	-	-	2	-	-	1
Co-Trimoxazole (25 µg)	1	-	22	1	-	4	1	-	1	-	-	1
Vancomycin (30 µg)	1	1	21	-	-	5	-	-	2	-	-	1
Teicoplanin (30 µg)	-	5	18	-	-	5	-	-	2	1	-	1
Ciprofloxacin (5 µg)	-	-	23	-	-	5	1	-	1	-	-	1
Gatifloxacin (5 µg)	1	2	20	1	1	3	-	-	2	-	-	1
Ofloxacin (5 µg)	1	-	22	1	-	4	-	-	2	-	-	1
Lomefloxacin (10 µg)	-	3	20	-	-	5	-	-	2	1	-	-
Norfloxacin (10 µg)	1	-	22	1	-	4	-	-	2	1	-	-
Moxifloxacin (5 µg)	2	-	21	1	-	4	-	-	2	-	-	1
Rifampicin (5 µg)	3	1	19	-	-	5	-	-	2	-	-	1
Tigecycline (15 µg)	7	-	16	2	-	3	1	-	1	-	-	1
Clindamycin (2 µg)	2	1	20	-	-	5	-	-	2	-	-	1
Novobiocin (30 µg)	3	-	20	-	-	5	-	-	2	-	-	1
Pristinamycin (15 µg)	4	2	17	1	-	4	1	-	1	-	-	1
Nitrofurantoin (300 µg)	4	6	13	1	3	1	1	1	-	-	1	-
Gentamicin (10 µg)	16	-	7	3	-	2	2	-	-	1	-	-
Cefoxitin (30 µg)	3	-	20	2	-	3	-	-	2	-	-	1
Ampicillin/Sulbactum (10/10 µg)	1	-	22	-	-	5	-	-	2	-	-	1
Piperacillin/Tazobactum (100/10 µg)	3	-	20	-	-	5	-	-	2	-	-	1

API-Staph Biomerieux identification system (APIS) and confirmed as coagulase-negative by the plasma-coagulase test. All the isolates were negative for coagulum formation.

The results of the susceptibility testing of the isolates to 24 antimicrobial agents showed a higher level of resistance against antibiotics like gentamicin (70.90%), azithromycin (64.50%), tigecycline (32.20%), nitrofurantoin (19.30%), streptogramin (19.30%) and cefoxitin (16.10%). Most of the isolates were susceptible to ciprofloxacin (96.70%), ampicillin/ sulbactam (96.70%) and fluoroquinolones (83-90%). The detailed phenotypic resistance profile including number of resistant (R), intermediately susceptible (I), and susceptible (S) isolates of CoNS from different seafood samples is shown in Table 2. Results showed that all the isolates were resistant to multiple antimicrobials.

DISCUSSION

Among Staphylococci, CoNS are a heterogeneous group of organisms divided into 40 species and 24 subspecies, classified into four representative groups: *S. epidermidis* group, *S. lugdunensis*, *S. saprophyticus* subsp. *saprophyticus*, and other CoNS (Becker *et al*., 2014). Among them, the "*S. epidermidis* group" was clinically defined as "medium pathogenic Staphylococci" and comprised of *S. epidermidis*, *S. haemolyticus*, *S. capitis*, *S. hominis*, *S. simulans*, and *S. warneri*. There have been many reports on the emerging pathogenicity of these microbes (Von Eiff *et al*., 2005). The incidence of multi-drug resistance in seafood samples adversely impacts human health as they cause opportunistic infections and Staphylococcal food poisoning. CoNS may be identified in dust and soil but are largely isolated from mammals where they are temporary or permanent inhabitants (Huebner and Goldmann,1999). Various reports are available worldwide regarding the pathogenicity and toxic production leading to the infection of humans (Breckinridge *et al*., 1971; Olsvik *et al*., 1982; Crass and Bergdoll, 1986; Kahler *et al*., 1986; Kloos and Bannerman, 1994; Vernozy-Rozand *et al*., 1996; Udo *et al*., 1999; Zell *et al*., 2008). The emergence of antibiotic-resistant CoNS has been recently noted in many food items such as milk, meat etc., but there is not much data on the prevalence of CoNS and its resistance pattern in the fish. In recent years, the number of literature on MDR bacteria in retail fish markets has increased since fish markets are considered a major source of MDR contamination. Generally, most of the CoNS contamination occurs through mishandling or post-harvest contamination. The food chain can become contaminated mainly during food processing, transportation or storage. In the present study, 31 MDR CoNS, including *S. warneri, S. haemolyticus, S. hominis, S. saprophyticus, S. simulans* and *S. xylosus* were found in retail fish markets of Veraval, Gujarat, with high levels of resistance to gentamicin (aminoglycoside), azithromycin (macrolide), and tigecycline (glycylcycline).

Aminoglycosides are one of the antibiotic classes that play an important role in the treatment of staphylococcal and enterococcal infections. The present study demonstrated a higher level of resistance (70.90%) to gentamicin, and it represents a serious compromise in the treatment strategy. The second-generation macrolides, azithromycin, have a broader spectrum of activity against Gram-positive bacteria. Thus, a higher level of resistance (64.40%) to azithromycin showed the ineffectiveness of this drug to that extent. Tigecycline is the minocycline derivative of class glycylcyclines with broad-spectrum activity. Results showed about 32.20% were categorized as tigecycline resistant. Linezolid and vancomycin resistance is uncommon compared to others, but resistance has begun to emerge. This poses a serious public health concern given the fact that they are considered a last-line resort for MDR gram-positive organisms. One isolate exhibited resistance to these two antibiotics. The most important

finding was that all the 31 CoNS isolates showed multi-drug resistance since they were resistant to more than three antibiotics classes. From the result, it can be emphasized that seafood items might be a vehicle for transmission of multidrug-resistant CoNS, as many countries used to eat seafood without processing (e.g. Sashimi). Thus, the scenario might be more critical if ready to eat food items become a source for multidrug-resistant bacteria. Still, the condition of the fish market has not been changed. A proper hygienic handling method is necessary for all fish vendors. A regular supply of potable water may reduce 80% of the MDR bacteria in the retail fish market.

The present study demonstrates an alarming presence of multi-drug resistant CoNS in seafood samples, and their quality can be compromised if good hygienic practices are not implemented. The results reveal a necessity for surveillance of identification coupled with antimicrobial resistance of CoNS in each stage of the food chain, from fishing to retail. Thus, fundamental Hazard Analysis and Critical Control Points are required to ensure seafood safety and hygiene.

This study confirms the presence of multi-drug resistant CoNS from the seafood samples of the retail market. Poor personal hygiene would be the major reason for the contamination of human-borne microbes including Staphylococci. The present study evaluated the presence of CoNS in seafood samples and emphasized the possible chance of transferring AMR to humans. Thus, regular monitoring and control over the food chain are required to minimize the presence or spread of drug-resistant bacteria to human consumers.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: GKS: Project PI, collected the samples, carried out the microbiologically screening and analysis and corrected the manuscript; AV: Drafted the manuscript and assisted lab works; VS: Carried out the lab work for the confirmation of Staphylococci; TM: Assisted with the lab work and molecular confirmation; MMP: Corrected the manuscript and interpreted the antibiogram data; CNR: Checking the manuscript and data interpretation.

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