

Incidence of coagulase negative Staphylococci and its AMR (antimicrobial resistance) level in seafood, Veraval, Gujarat

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The isolation of coagulase negative Staphylococci (CoNS) from 22 number of seafood samples consisting of lobster (3), squid (3), shrimps (2), octopus (3), ribbon fish (4), tuna (2), surimi (3) and cobia (2), Veraval region, Gujarat, was done on the basis of colony morphology on Baird Parker Agar (Oxoid) supplemented with 5% egg yolk emulsion and 1% potassium tellurite solution with 48 h incubation at 35°C ± 2°C as per the ISO 6888, 2003. Well isolated colonies were streaked on to Tryptic Soy Agar (TSA) for further purification and identification. Thirty one isolates of CoNS were identified as *S. haemolyticus* (6), *S. saprophyticus* (3), *S. hominis* (4), *S. simulans* (3), *S. warneri* (12) and *S. xylosus* (3) by Staph Biomerieux identification system. The distribution of CoNS from the collected fish samples were *S. haemolyticus* (2), *S. warneri* (3) and *S. saprophyticus* in lobsters; *S. simulans*, *S. warneri* and *S. xylosus* in squids; *S. haemolyticus*, *S. saprophyticus* and *S. warneri* (3) in shrimps; *S. hominis* and *S. simulans* in octopus; *S. haemolyticus* (2), *S. saprophyticus*, *S. warneri* (2) and *S. xylosus* in ribbon fish; *S. hominis*, *S. simulans* and *S. warneri* in surimi, and *S. hominis* and *S. warneri* in the cobia samples. The antimicrobial sensitivity test to 24 antibiotics viz., Penicillin-G (P) 10 µg Azithromycin (AZM) 15µg, Erythromycin (E) 15µg, Clarithromycin (CLR) 15µg, Linezolid (LZ) 30µg, Co-Trimoxazole (COT) 25µg, Vancomycin (VA) 30µg, Cefoxitin (CX) 30µg, Ciprofloxacin (CIP) 5µg, Gatifloxacin (GAT) 5µg Ofloxacin (OF) 5µg, Clindamycin (CD) 2µg, Tigecycline (TGC) 15µg, Moxifloxacin

(MO)5µg, Gentamicin (GEN)10µg, Rifampicin (RIF)5µg, Lomefloxacin (LOM)10µg, Norfloxacin (NX)10µg, Novobiocin (NV)30µg, Teicoplanin (TEI) 30µg, Nitrofurantoin (NIT) 300µg, Pristinomycin (RP) 15µg Ampicillin/Sulbactam (A/S) 10/10µg, Piperacillin/Tazobactam (PIT) 100/10µg (Dodeca Staphylococci-1 and 2, HiMedia, Mumbai) were carried out by disc diffusion method (Kirby-Bauer, 1966) on Mueller Hinton agar with 4% NaCl and incubated at 37°C for 18- 24 h. The inhibition zones were measured and evidenced as sensitive, intermediate and resistant as per CLSI breakpoints (2015) and all of antibiotics concentrations used were as per the CLSI recommendations. The MIC levels were determined with MIC detection strip for the antibiotics such as Methicillin A (0.01-240 µg/ml), Methicillin B (0.001-4 µg/ml), Penicillin (0.002-32 µg/ml), Oxacillin (0.016-256 µg/ml) Vancomycin (0.016-256 µg/ml), Gentamicin (0.016-256 µg/ml) and Ciprofloxacin (0.002-32). CoNS isolates showed higher level of resistance against gentamicin (70.9%), azithromycin (64.5%), vancomycin (45.1%), tigecycline (32.2%), and nitrofurantoin (19.3%). Intermediate level of resistance was also found with vancomycin (41.9%), nitrofurantoin (35.4%) and erythromycin (22.5%). Maximum CoNS isolates were susceptible to novobiocin (100%) followed by ciprofloxacin (96.7%), ampicillin/sulbactam (96.7%) and fluoroquinolones (83-90%)(Table). The MIC levels was found highest with oxacillin (51.61%), ciprofloxacin (38.71%), amoxyclav (35.48%) followed by erythromycin (6.45%) and clindamycin (3.23%) and methicillin

(3.23%). The intermediate resistant were also found among these isolates with vancomycin (38.71%), erythromycin (12.9%) and tetracycline (3.23%). The average MIC values for oxacillin, amoxyclav, clindamycin, gentamycin, methicillin, tetracycline, ciprofloxacin, erythromycin and vancomycin were 12.84, 7.12, 0.41, 1.16, 5.11, 0.21, 3.83, 16.24 and 2.48 µg/ml, respectively.

The presence of antimicrobial resistance in CoNS is mainly due to the commonly used antimicrobial agents such as oxacillin, ciprofloxacin, erythromycin, clindamycin and methicillin for the treatment of Gram's positive bacteria. This could pose public health threat and it may act as a reservoir for the horizontal transfer of antimicrobial resistance.

Table: Antimicrobial resistance pattern of CoNS from the seafood samples.

Sl. No	Name of the antibiotic	Antibiotic group	Resistant (%)	Intermediate susceptibility (%)	Susceptible
1	Penicillin (P)	Penicillin	19.3	0	80.6
2	Erythromycin (E)	Macrolides	9.6	22.5	67.7
3	Clarythromycin (CLR)		9.6	3.2	87.09
4	Linezolid (LZ)	Oxazolidinones	9.6	0	90.3
5	Co-Trimoxazole (Trimethoprim/Sulphamethaxole (COT))	Sulfonamides	9.6	0	90.3
6	Azithromycin (AZM)	Azalides	64.5	0	35.4
7	Ciprofloxacin (CIP)	Floroquinolones	3.2	0	96.7
8	Gatifloxacin (GAT)		6.4	9.6	83.8
9	Ofloxacin (OF)		6.4	0	93.5
10	Lomefloxacin (LOM)		3.2	9.6	87.09
11	Moxifloxacin (MO)		9.6	0	90.3
12	Norfloxacin (NX)		9.6	0	90.3
13	Clindamycin (CD)	Lincosamide	6.4	3.2	90.3
14	Cefoxitin (CX)	Cephems	16.1	0	83.8
15	Vancomycin (VA)	Aminoglycosides	45.1	41.9	12.9
16	Gentamicin (GEN)		70.9	0	29.03
17	Nitrofurantoin (NIT)		19.3	35.4	45.1
18	Rifampicin (RIF)	Rifamycin	9.6	3.2	87.09
19	Tigecycline (TGC)	Glycylcyclines	32.2	0	67.7
20	Teicoplanin (TEI)	Glycopeptides	3.2	16.1	80.6
21	Novobiocin (NV)	Aminocoumarin	0	0	100
22	Pristinamycin (RP)	Streptogramin	19.3	6.4	74.1
23	Ampicillin/Sulbactam (A/S)	Beta-lactam & Beta-lactamase inhibitors	3.2	0	96.7
24	Piperacillin/Tazobactam (PIT)		9.6	0	90.3