Proskauer negative, glucose fermenters, lactose fermenters and non-citrate utilizers. Antibiotic resistance in these 31 isolates were tested simultaneously using the standard agar disc diffusion method (CLSI, 2012) using Mueller-Hinton agar. All these isolates were grown overnight in Tryptic soya broth at 37 °C and adjusted to 0.5 McFarland Standard. The multidisc (Icosa G-II-Minus, Himedia, India) used in the study contained the following antibiotics arranged equidistant to each other: Imipenem (IPM) - 10µg; Ciprofloxacin (CIP) - 5µg; Tobramycin (TOB) - 10µg; Moxifloxacin (MO) - 5µg; Ofloxacin (OF) - 5µg; Ceftazidime (CAZ) - 30µg; Levofloxacin (LE) - 5µg; Norfloxacin (NX) - 10µg; Co-Trimoxazole (COT) - 25µg; Colistin (CL) - 10µg; Nalidixic acid (NA) - 30µg; Augmentin (AMC) - 30µg; Cefoxitin (CX) - 30µg; Gatifloxacin (GAT) - 5µg; Gentamicin (GEN) - 10µg; Amikacin (AK) - 30µg; Aztreonam (AT) - 30µg; Ceftriaxone (CTR) - 30µg; Cefpodoxime (CPD) - 10µg and Nitrofurantoin (NIT) - 300µg. The plates were incubated at 37 °C for 18 h. The diameters of inhibition zones were measured in millimetre, and interpreted in accordance to CLSI recommendations. Among the 20 antibiotics tested against 31 isolates, 15 isolates showed resistance to Imipenem (IPM) with 48.38%, 12 isolates showed resistance to Nitrofurantoin (NIT) with 38.70% and 11 isolates showed resistance to Cefpodoxime (CPD) of 35.48% and eight isolates showed resistance to Nalidixic acid (NA) of 25.8%. None of the isolates showed resistance to Norofloxacin (NX), Levofloxacin (LE), Tobramycin (TOB), Amikacin (AK) and Cefoxitin (CX). The results are shown in (Fig. 1). Six out of 31 isolates have shown multi-drug resistance to more than three classes of antibiotics (16.1%). Multi-drug resistance in pathogenic bacteria is an universal problem across the globe and is prevailing in many fields of science. Therefore, strict awareness, measures and regulations are to be standardized for use in seafood production to combat this problem.

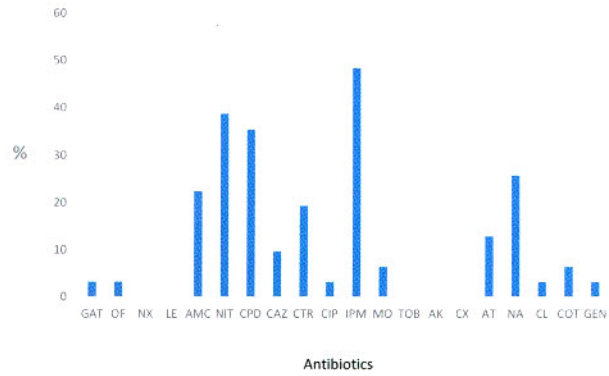

Fig. 1. Antibiotic resistance pattern of *E. coli* Isolates

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Isolation and antibiotic resistance pattern of Staphylococci from seafood of Veraval, Gujarat

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Staphylococci are commonly associated with the skin of the food handlers which can act as a source of food contamination and is considered as a versatile human pathogen. In the recent years it has emerged as a major and most difficult pathogen to treat due to the resistance developed

against several antibiotics especially to Methicillin. They are also responsible for many of the community-acquired diseases and nosocomial infections in humans. They have been widely studied from livestock farms and food animals, meat and poultry products, milk and livestock workers, and fish handlers. The presence of antibiotic-resistant Staphylococci was well studied in cultured fish, fresh seafood, ready-to-eat and ready-to-cook fish products, seafood processing environments and ice. There are several factors responsible for the spread of these bacteria such as poor maintenance of hygiene and sanitation conditions by the fish handlers cross contamination during handling and storage. The antibiotic resistant pattern of *S. aureus* isolated from seafood was reported by Murugadas *et al.*, 2016.

This article reports the studies on screening of seafood samples for the presence of Staphylococcus from Veraval coast. A total of 17 *Staphylococcus* sp. were isolated from fish and shellfish products. The isolation was carried out as per the United States Food and Drug Administration, Bacteriological Analytical Manual (Bennett and Lancette, 2016). The biochemical characterization was carried out as per the standard procedures described in Bergey's Manual of Systemic Bacteriology (2005). All the 17 isolates were Gram positive cocci shape, catalase positive, oxidase negative and coagulase positive. The antibiotic resistance pattern was studied against 24 antibiotics belonging to more than nine major groups. The isolates which are resistant and sensitive to the tested antibiotics are given in Table 1. This indicates high percentage of resistance for Penicillin G (64.7%) followed by

Table 1. Percentage of resistant and sensitive *Staphylococcus* sp. isolated from seafood

Antibiotic and Concentration	Major group of antibiotic	Resistant		Sensitive		MDR (More than 3 classes)
		No. of isolates	%	No. of isolates	%	
1. Penicillin G (100U)	Penicillin	11	64.7	6	35.3	9 (52.9%)
2. Azitrhomycin (15 µg)	Macrolides	6	35.3	11	64.7	
3. Erythromycin (15 µg)	Macrolides	6	35.3	11	64.7	
4. Clarithromycin (15µg)	Macrolides	5	29.4	12	70.6	
5. Linezolid (30 µg)	Oxazolidinones	1	5.8	16	94.2	
6. Co-Trimoxazole (25 µg)	Sulphonamide	0	0	17	100	
7. Vancomycin (30 µg)	Glycopeptides	0	0	17	100	
8. Cefoxitin (30 µg)	Cephalosporin	2	11.7	15	88.3	
9. Ciprofloxacin (5 µg)	Quinolones	3	17.0	14	83.0	
10. Gatiflaxacin (5 µg)	Quinolones	3	17.0	14	83.0	
11. Ofloxacin (5 µg)	Quinolones	1	5.8	16	94.2	
12. Clindamycin (2 µg)	Lincosamides	4	23.5	13	76.6	
13. Tigecycline (15µg)	Tetracycline	0	0	17	100	
14. Moxifloxacin (5µg)	Macrolides	1	5.8	16	94.2	
15. Gentamicin (10µg)	Aminoglycosides	1	5.8	16	94.2	
16. Rifampicin (5 µg)	Ansamycins	0	0	17	100	
17. Lomefloxacin (10µg)	Quinolones	8	47.0	9	53.0	
18. Norfloxacin (10µg)	Quinolones	0	0	17	100	
19. Novobiocin (30 µg)	Aminocoumarin	0	0	17	100	
20. Teicoplanin (15 µg),	Glycopeptides	0	0	17	100	
21. Nitrofurantoin (300 µg)	Nitrofurans	1	5.8	16	94.2	
22. Pristinnomycin (15 µg)	Streptogramin	7	41.0	10	59.0	
23. Ampicillin-Sulbactum (10/10 µg)	Penicillin	3	17.0	14	83.0	
24. Piperacillin- Tazobactum (100/ 10 µg)	Penicillin	10	58.8	7	42.2	

Piperacillin/Tazobactam (58.8%). No resistance was observed for Co-Trimoxazole, Vancomycin, Tigecycline, Rifampicin, Norfloxacin, Novobiocin and Teicoplanin. The percentage of antibiotic resistance of the *Staphylococcus* sp. isolated from seafood is shown in Fig. 1.

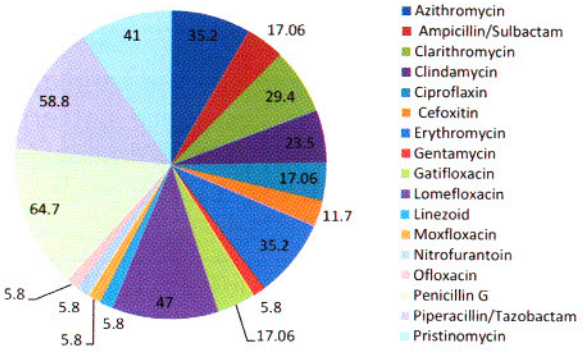

Fig.1 Percentage of antibiotic resistance of *Staphylococcus* sp. isolated from seafood of Veraval

The antibiotic resistant profiles were carried out using disc diffusion method (CLSI, 2014) on Mueller-Hinton agar. The overnight grown cultures (Tryptic Soy broth, BD Difco, India) was centrifuged (Heraeus Kendro: Biofuge Stratus, UK) at 2,500 xg for 30 min. at 4 °C and the pellet was dissolved in normal saline. The optical density was adjusted to 0.5 McFarland standard and spread onto the agar plates. Further, the antibiotics (Staphylococci I and Staphylococci II disc, Himedia, India) were placed on to the plate and were incubated at 37 °C for 16-20 h. The antibiotics used and their concentrations are provided in Table 1. The diameter of the inhibition zones were measured in millimetre and interpreted in accordance with CLSI guidelines. The resistance profile of the 17 isolates against the tested antibiotics is given in Table 2. Out of 17 isolates, nine exhibited multi-drug resistance (MDR) to the tested. The MDR rate is 52.9%. Among nine multi-drug resistant isolates, two were resistant to Cefoxitin which are multi-drug resistant Methicillin resistant *Staphylococcus aureus* (MRSA). The high prevalence of multi-drug resistant *Staphylococcus* sp. in the fishery products

Table 2. Antibiotic resistance profiles of *Staphylococcus* sp. isolated from seafood

Sl No	Culture	Resistance pattern	Class of antibiotic
1	S1	LOM	Q
2	S2	P LOM LZ	P Q O
3	S3	P PIT RP AZM E CLR	PP S MMM
4	S4*	P PIT RP AZM E CLR CD A/S CX	PP S MMM L P C
5	S5	Sensitive to all classes	
6	S6	LOM CIP GAT	QQQ
7	S7	P PIT RP AZM CLR GEN NIT	PP S MM A N
8	S8	LOM CD GAT	Q L Q
9	S9*	P PIT RP E CD A/S CX	PP S M L P C
10	S10	P PIT LOM CIP	PP Q Q
11	S11	PIT LOM CIP GAT	P QQQ
12	S12	P PIT RP AZM E CLR	PP S MMM
13	S13	MO	Q
14	S14	P PIT RP AZM A/S	PP S M P
15	S15	P PIT RP AZM E CLR	PP S MMM
16	S16	P PIT LOM CD OF	PP Q L Q
17	S17	P LOM	P Q

(S-*Staphylococcus* sp. LOM - Lomefloxacin; P - Penicillin G; LZ - Linezolid; PIT - Piperacillin - Tazobactum; RP - Pristionomycin; AZM - Azitrhomycin; E - Erythromycin; CLR -Clarithromycin; CD - Clindamycin; A/S - Ampicillin - Sulbactum; CX - Cefoxitin; CIP -Ciprofloxacin; GAT - Gatiflaxacin; GEN - Gentamicin; NIT - Nitrofurantoin; MO -Moxifloxacin; OF - Ofloxacin; Q - Quinolones; P - Penicillins; O - Oxazolidinones; S -sulphonamide; M - Macrolides; L - Lincosamides; C - Cephalosporins; A - Aminoglycosides; N - Nitrofurans *Presumptive MRSA isolates)

is of high concern in terms of consumer health. Therefore, awareness is to be created among the food handlers about the cleanliness and the measures to be taken to reduce the prevalence of this hazardous bacterial pathogen.

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Evaluation of dry rehydratable film (3M™ Petrifilm™) method for microbial enumeration in fish samples

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Petrifilms eliminate the time for media preparation and sterilization.The 3M™ Petrifilm™ is an all-in-one plating system. Rather than a petridish, 3M™ Petrifilm™ makes use of a thin plastic film as carrier of the culture medium. 3M™ Petrifilm™ comprises a cold-water-soluble gelling agent, nutrients and indicators for activity and enumeration (Jasson *et al.*, 2010). Day by day developing food testing sector provides alternatives to existing standard methods. Before adopting novel techniques it is important to evaluate the effectiveness of the method. In this backdrop, a study was carried out to evaluate the applicability of the petrifilm method for enumeration of aerobic microorganisms, Enterobacteriaceae, *S.aureus*, *E. coli* and Coliforms by comparing the results of petrifilm method with that of standard enumerating techniques. Evaluation of dry rehydratable film (3M™ Petrifilm™) method for microbial enumeration in seafood samples is depicted in Fig.1.

Seven seafood samples were analyzed for microbial parameters like Aerobic Plate Count, Enterobacteriaceae, *Escherichia coli*, Total Coliforms and *Staphylococcus aureus*. Analysis were performed using petrifilms and standard conventional agar plates in triplicate (n=3).The fish *Elagatis bipinnulata*, *Caranx* spp., *Scomberomorus commerson* and *Lethrinus lentjan* were collected from Cochin Fisheries Harbour. *Fenneropenaeus indicus* (headless), *Thunnus albacores* (loin) and *Octopus vulgaris* (Baby octopus) were obtained from a processing plant in Cochin.

Petrifilms were developed in the early 1980s for enumerating aerobic bacteria in food samples. Petrifilm™ Aerobic count plates have an indicator dye and built in grid allows for fast and accurate identification of colonies, within 48 hours over a growth area of approximately 20 cm^2. The comparison of mesophilic count of fish samples with Petrifilm method against the pour plate method showed a significant correlation ($r=0.991$, $p < 0.05$). The reduction of the dye in the upper film of the plates gives red colour to aerobic bacterial colonies. Linton *et al.* (1997) reported that in conventional media certain mesophilic