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Antimicrobial susceptibility pattern of staphylococcus aureus isolated from milk and milk products

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Abstract
The incidence of Staphylococcus aureus infections are being common in veterinary field. This study was an attempt to know the antibiotic sensitivity pattern of the *S. aureus* in the samples collected from Hyderabad. Samples comprising were 240 (30 each of raw milk, pasteurized milk, paneer, cheese, curd, khova, ice cream and pedha) samples, from which the colonies were isolated and grown in *S. aureus* selective mannitol salt agar plates. The antimicrobial sensitivity pattern of seven antibiotics was evaluated in this study. The antibiotics selected for the study were erythromycin (15 µg), Pencillin-G (25 µg), Ampicillin (10 µg), Oxacillin (1 µg), Ciprofloxacin (5 µg), Gentamycin (10 µg), Methicilin (10 µg), Streptomycin (10 µg), Vancomycin (30 µg) and Tetracyclin (30 µg). The maximum inhibitory zone was shown by Gentamycin (88%), followed by Ciprofloxacin (86%) and Erythromycin (64%) and the resistance was the maximum towards Pencillin G (98%) and Ampicillin (88%). This organism was intermediately resistant to Vancomycin (26%) and Streptomycin (14%). Hence it could be concluded that Gentamycin is the best cost effective drug with maximum inhibitory zone against *S. aureus* infections among the nine antibiotics studied.

Keywords: antimicrobial susceptibility, staphylococcus aureus, milk and milk products

Introduction
S. aureus is Gram positive cocci which is a facultative anaerobe. *Staphylococcus* was first identified in 1880 in Aberdeen, Scotland, by the surgen Sir Alexander Ogston in pus from a surgical abscess in a knee joint. It is commonly present on skin flora and in nasal passage. It is one of the most common causes of nosocomial infections and can also cause post-operative infections (Shorr *et al.*, 2005) [28]. *S. aureus* is coagulase-positive; having golden yellow colonies. Local purulent infections: furuncles, carbuncles, bullous impetigo, wound infections, sinusitis, otitis media, mastitis puerperalis, ostitis, post influenza pneumonia, sepsis. Toxins produced by *S. aureus* cause food poisoning, dermatitis exfoliativa and toxic shock syndrome.
Staphylococcus aureus is considered the third most important cause of disease in the world among the reported food borne diseases (FBDs). A wide variety of foods support the growth of *S. aureus* and are ideal for enterotoxin production including: raw milk, dairy products, meat, meat products and ready-to-eat foods (Aydin *et al.*, 2011) [4].The growth of *S. aureus* in foods may pose a potential public health hazard because many strains of *S. aureus* produce enterotoxins (SEs) which are the causative agents of staphylococcal food poisoning (SFP) (Peles *et al.*, 2007) [23].
SFP presents as a self-limiting gastrointestinal illness with emesis following a short incubation period (4hours or some times as low as so) after ingestion of food containing preformed enterotoxin(s). Vomiting is accompanied by other symptoms i.e. nausea, diarrhea, abdominal pain (Alouf *et al.*, 2003) [3].
Out of 24 different SEs that have been described, SEA, SEB, SEC, SED, and SEE are considered the classical SEs that have been studied and are better understood than the newly described SEs (SEG-SEU) (Sharma *et al.* 2000). TSST-1 and the staphylococcal enterotoxins are also known as pyrogenic toxin super-antigens (PTSAgs) (Dinges *et al.*, 2000) [10]. SEA and SEB are the most common SEs which can cause disease in very small amounts i.e. 100-200 ng/ml or 10-20 ng/ml respectively (Clarisse *et al.*, 2013) [6]. So rapid and sensitive detection is needed in order to diagnose the illness or identify the contaminated food correctly.
Antibiotic resistance leads to prolonged hospital stay, contaminated milk and milk products and increased costs in terms of treatment. In addition to these, it causes life threatening infections such as in cases of pyomyositis and chronic osteomyelitis. The majority of the MRSA strains worldwide have become resistant to multiple antibiotics including beta-lactams;

tetracyclines, macrolides and more recently fluoroquinolones. Excessive use of penicillin antibiotics over the years has led to the development of resistant strains of bacteria that are no longer killed by other beta lactam antibiotics.

Antimicrobial agents are among the most commonly used and misused of all drugs. The inevitable consequence of the widespread use of antimicrobial agents has been the emergence of antibiotic resistant pathogens, fueling an ever increasing need for new drugs. However, the pace of antimicrobial drug development has slowed dramatically, with only a handful of new agents, few of which are novel, been introduced into clinical practice each year. Reducing the inappropriate antibiotic use is thought to be the best way to control resistance (Cookson and Phillips, 1998) [7].

First identification and isolation of the causative organism should be taken place in the microbiology laboratory. Once the microbial species causing the disease have been identified, a rational choice of the class of antibiotics likely to work in on the patient can be made (Henry, 2010) [16].

Materials and Methods

Isolation and Identification

First step done was the collection of samples, milk samples each of 50 ml were aseptically collected from local vendors, college farm and local private dairy farms, milk products from different parlours of co-operative and private sector dairies and local sweet shops in sterile vials. The samples were immediately brought to the laboratory after collection. In the laboratory the samples were inoculated in 7% sodium chloride solution and incubated at 37°C overnight. Later the prepared samples were subcultured on mannitol salt agar plates. The milk samples were directly inoculated into mannitol salt agar plates and incubated for 24 hours at 37°C. The presumptive colonies were picked up and were subjected to biochemical tests. The isolates were identified with standard tests used to identify *S. aureus* such as Gram stain, catalase and coagulase tests. After this, sample was inoculated on plates by four flame method and incubated at 37°C for 24 hours (Forbes, 2007) [13].

Antimicrobial susceptibility tests

Antibiotic sensitivity was performed by Disc Diffusion Method of Bauer *et al.* (1966) [5]. MH broth was inoculated with five colonies from plates and tubes were incubated at 37°C for 2-8hrs until achieving a turbidity equivalent to 0.5 on the Macfarlandt scale. After turbidity adjustment a sterile swab was introduced, pressed against the tube well in order to remove any excess liquid; and then seeded on the surface of a petri dish containing MH agar, rotating atleast twice. After the liquid was placed the disc was left at rest for five minutes to absorb any excessive humidity (Kumar and Kalpana, 2013) [21] Then same commercially available antibiotic discs were gently and firmly placed on the agar plates, which were then left at room temperature for 1 hour to allow diffusion of the antibiotics into the agar medium. The plates were then incubated at 37°C for 24 hours. Filter paper discs containing designated amount of the antimicrobial drugs obtained from commercial supply firms (Himedia Labs, Mumbai, India) were used. Antimicrobial susceptibility isolates was established by the disc diffusion assay with Muller- Hinton agar in accordance with French National antibiogram committee guidelines.

The antibiotic sensitivity of S.aureus was tested for antibiotics such as erythromycin (15 µg), Pencillin-G (25 µg), Ampicillin (10 µg), Oxacillin (1 µg), Ciprofloxacin (5 µg), Gentamycin (10 µg), Methicilin (10 µg), Streptomycin (10 µg), Vancomycin (30 µg) and Tetracyclin (30 µg). The antimicrobial activity was present on the plates, was indicated by an inhibition zone. The diameter of the inhibition zones was measured in millimeter after 24 hours using a scale. An organism was interpreted as highly susceptible if the diameter of inhibition zone was more than 19 mm, intermediate if diameter was 15-18 mm and resistant if the diameter was less than 13 mm. The intermediate readings were considered as sensitive in the assessment of the data.

Results and Discussion:

240 milk and milk products samples tested, organisms isolated were identified as *Staphylococcus aureus*. The colonies were gram positive cocci, non-motile, non-capsular and non-sporulating organisms. They were tend to occur in irregular clusters resembling bunches of grapes. The coagulase test was positive and with golden yellow colonies.

Percentage of antibiotic sensitivity ranged between 25-80 % (Table-1), for Gentamycin. Among them, Gentamycin was with highest inhibitory zone, followed by Ciprofloxacin and Erythromycin. The intermediate sensitivity was exhibited to Vancomyci and Streptomycin discs. The inhibitory zone was least for Pencillin-G.

The sensitivity of *S.aureus* to Gentamicin (88.0%) in the present study was less than the sensitivity of 100% (Suleiman *et al*., 2013) [30], 91.7% (Aber *et al*., 2010) [1], 90.0% (Beyene, 2016 and Thaker *et al.*, 2013) [31] and more than the resistance of 85.83%, 82.8%, 32.0% and 5.7% reported by Nazeer Naher Islam (2014), Owuna *et al.* (2012), Brinda *et al.* (2010) and Seedy *et al.* (2010) [25] respectively. The resistance to Gentamicin was 4.0% in the present study, was less than the resistance (82.8%, 75.0%, 67.4%, 34.6%, 30.5%, 22.22%, 19.8% and 10.0%) reported by Owuna *et al.*(2015) [22], Jabber *et al.* (2011), Zouhairi *et al.* (2010) [33], De Medeiros *et al.* (2011) [9], Kumar *et al.* (2009), Sharma *et al.* (2015) [26], Lubna *et al.* (2010) and Thaker *et al.* (2013) [31] respectively. Very low resistance of 3.8% and 3.0% was reported by Seedy *et al.* (2010) [25] and Pu *et al.* (2011).

The resistance of *S.aureus* to Pencillin-G in the present study (98.0%) was more than the resistance (72.0%, 64.0% and 22.7%) reported by Shitandi and Milcah (2004), Seedy *et al.* (2010) [25] and Kumar *et al.* (2009) respectively. Higher resistance to Pencillin-G (100%) was reported by Beyene *et al.* (2016) and Thaker *et al.* (2013) [31]. An Intermediate resistance (2.0%) observed in the present study was less than 17.1% and 7.5% reported by Shitandi and Milcah (2004) and Seedy *et al.* (2010) [25] respectively.

The resistance of *S.aureus* to Tetracyclins was 46.0% in the present study, which was more than the resistance (42.85%, 37.9%, 36.7%, 30.0%, 26.1%, 22.22% and 11.0%) reported by Elbargisy *et al.* (2016), Shitandi and milcah (2004), kumar *et al.* (2009), Jabber *et al.* (2011), Alian *et al.* (2012), Sharma *et al.* (2015) [26] and Spanu *et al.* (2010) respectively, whereas higher resistance (100%, 67.0%, 59.2%, 58.7% and 52.8%) was reported by Yurdakul *et al.* (2013), Pu *et al.* (2011), De Medeiros *et al.* (2016), Lubna *et al.* (2015) and Elbargisy *et al.* (2016) respectively. The sensitivity of present study (46.0%) was almost similar to the sensitivity (45.0%) reported by Jaber *et al.* (2011) [14], whereas lower (17.6%) sensitivity was reported by Shitandi and Milcah (2004). Intermediate resistance (8.0%) observed in the present study was more than 25.0% and 24.5% reported by Jabber *et al.* (2011) and

Shitandi and Milcah (2004).

The sensitivity of *S.aureus* to Ciprofloxacin in the present study (86.0%) was more than the sensitivity (83.33%, 83.0%, 80.0%, 35.0% and 10.43%), which was reported by Jahan *et al.* (2015), Seedy *et al.* (2010) [25], Thaker *et al.* (2013) [31], Jaber *et al.* (2011) [14] and Nazneen Nahan Islam *et al.* (2014) respectively. The resistance to Ciprofloxacin (14.0%) in the present study was less than the resistance of 77.5%, 45.2% and 22.22% reported by Nazeer Naham Islam (2014), De Medeiros *et al.* (2016) and Sharma *et al.* (2015) [26] respectively. The resistance in this study was almost similar to the resistance of 13.0% reported by Pu *et al.* (2011). Lower resistance (7.5%) was reported by Seedy *et al.* (2010) [25]. An Intermediate resistance (8.0%) observed in the present study was less than 25.0% and 24.5% reported by Jabber *et al.* (2011) and Shitandi and Milcah (2004) respectively.

The resistance of *S.aureus* to Oxacillin was 68.0% in the present study, which was less than the resistance 100%, 93.5% and 71.4%) reported by Ugwu *et al.* (2015), Zouhairi *et al.* (2010) [33] and Pesavento *et al*. (2005) respectiely. Higher resistance than the present study (65.8%, 60.3%, 52%, 28.3%, 5.0% and 3.0%) was reported by De Medeirs *et al.* (2011), Daka *et al.* (2012), Brinda *et al.* (2010), Alian *et al.* (2012), Nemati *et al.* (2008), Abdel Halem *et al.* (2016) and Moon *et al.* (2007) respectively.

The resistance of *S.aureus* to Ampicillin was 88.0% in the present study, which was less than the resistance (100%, 96.0%, 91.1%, 68.0% and 42% reported by Nazeer Naham Islam (2014), Beyene (2016), Lubna *et al.* (2015), Pu *et al.* (2011) and Elbargisy *et al.* (2016) respectively. Lower resistance (40.0%, 36.1%, 33.33%, 25.97%, 20.8% and 16.0%) was reported by Thaker *et al.* (2013) [31], Spanu *et al.* (2010), Sharma *et al.* (2015) [26], Farzana *et al.* (2004), Seedy *et al.* (2010) [25] and Haran *et al.* (2012), respectively. The sensitivity in the present study (64.0%) was more than the sensitivity (61.6%, 55.0%, 49.0%, 41.4%, 32.1% and 14.17%) reported by Shitandi and Milcah (2004), Jabber *et al.* (2011), Seedy *et al.* (2010) [25], Owuna *et al.* (2015) [22], Daka *et al.* (2012) and Nazeen Nahar Islam *et al.* (2014) respectively. Higher sensitivity of 96.10% was reported by Farzana *et al.* (2004).

The resistance of *S.aureus* to Streptomycin was 70.0% in the present study, was almost similar to the resistance (71.7%) reported by Seedy *et al.* (2010) [25]. Lower resistance of 60.0%, 44.44%, 38.1%, 26.6%, 10.0% and 5.6% was reported by Achi and Madubuike (2007), Sharma *et al*. (2015) [26], De Medeiros *et al.* (2011) [9], kumar *et al.* (2009), Thaker *et al.* (2013) and Aber *et al.* (2010) [1] respectively. The sensitivity in the present study (16.0%) was less than the sensitivity (94.0%, 86.1%, 79.3% and 60.0%) was reported by Beyene (2016), Aber *et al.* (2010) [1], Owuna *et al.* (2015) [22] and Thaker *et al.* (2013) [31] respectively.

The resistance of *S.aureus* to Vancomicin (64.0%) in the present study was less than the resistance (90.0%, 88.89% and 71.7%) reported by Jabber *et al.* (2011), Sharma *et al.* (2015) [26] and Zouhairi *et al.* (2010) [33] respectively, whereas Lower resistance (25.7%) was reported by Lubna *et al.* (2015). The sensitivity of the present study (10.0%) was less than the sensitivity (38.5%) reported by Daka *et al.* (2012).

The resistance of *S.aureus* to Methicillin was 76.0% in the present study, which was less than the resistance (84.8%) reported by Zouhairi *et al.* (2010) [33]. Lower resistance (66.67%, 7.8% and 3.8%) was reported by Sharma *et al.* (2015) [26], Shitandi and Milcah (2004) and Seedy *et al.* (2010) [25] respectively. The sensitivity in the present study (16.0%) was less than the sensitivity (100.0% and 98.2%) reported by Thaker *et al.* (2013) [31] and Seedy *et al.* (2010) [25]

Table 1: Antimicrobial sensitivity of S. aureus isolated from milk and milk products

S. No	Antimicrobial agent	Concentration (µg)	Pattern of antibiogram		
			Sensitive (%)	Intermediate (%)	Resistant (%)
1	Erythromycin	(30 µg)	32(64%)	12(24%)	6(12%)
2	Gentamycin	(10 µg)	44(88%)	4(2%)	2(4%)
3	Pencillin –G	(10U)	-	1(2%)	49(98%)
4	Tetracycline	(30 µg)	23(46%)	4(8%)	23(46%)
5	Ciprofloxacin	(5 µg)	43(86%)	0	7(14%)
6	Oxacillin	(1µg)	11(22%)	5(10%)	34(68%)
7	Ampicillin	(10µg)	4(4%)	4(8%)	44(88%)
8	Streptomycin	(10µg)	8(16%)	7(14%)	35(70%)
9	Vancomycin	(10µg),	5(10%)	13(26%)	32(64%)
10	Methicilin	(30µg)	8(16%)	4(8%)	38(76%)

Conclusion

From the present study it could be concluded that Gentamycin is the best antibiotic of choice, among the seven antibiotics studied against *S. aureus* infection, with the highest inhibitory zone and the cost effective of therapy.

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