PUBLICATIONS

Papers published:

- **Sinha P**, Pandey P, Das R, Kar D. (2015). "Incidence of Methicillin Resistance *Staphylococcus aureus* in environmental sources from semi-urban locality in Assam." *Int. J basic and life sc.* 3(2): 9-15.

- Das R, **Sinha P,** Pandey P, Kar D. (2014). "Prevalence of MBL producing *Pseudomonas sp.* From soil- A case study in Assam University campus." Global Adv. Res. J Microbiol. 3(6): 098-101.

Papers communicated:

- Mauscript no. GARJM-15-011 entitled "A review on virulance factors of *Staphylococcus aureus*- the cause behind its pathogenecity" communicated in global advanced research journal of microbiology.

- Mauscript entitled "Haemolysin gene profiling of *Staphylococcus aureus* isolated from food samples in South Assam." recently communicated in Food Security journal.

- Mauscript entitled "Evaluation of endogenous virulence factors and antimicrobial resistance profile of *Staphylococcus aureus* in relation with small colony variants isolated from human clinical samples" recently communicated in annals of biology.

Incidence of Methicillin Resistance *Staphylococcus aureus*in environmental sources from semi-urban locality in Assam

Papiya Sinha[1], Piyush Pandey*[1], Ruchira Das[1] and Devashish Kar[2]

Abstract: *This study aimed to determine the presence of Methicillin Resistant Staphylococcus aureus (MRSA) on food contact surfaces in dairy, and meat environments . A total of 70 isolates were isolated using standard microbiological and biochemical methods (API Staph) from 127 environmental samples. The prevalence of MRSA was confirmed in 26.67% of samples. Antimicrobial susceptibility of isolates was determined by the Kirby-Bauer disk diffusion assay. Results were analyzed by the chi-square test and p values < 0.05 were considered significant. Although vancomycin was found 100% sensitive, marked resistance was observed for penicillin (87%), cefuroxime (66.66%) erythromycin (53.33%) ceftadizime (49%), gentamicin (47%). ciprofloxacin (42%), amikcin (31.6%). Uneven microbiological conditions were found in each food zone, which indicates the need to improve hygienic conditions as these food surfaces and its surrounding environments could thus be a reservoir for S. aureus forming complex communities. Therefore, foodstuff and its adjoining atmosphere should be properly managed to enhance the safety of food products.*

Key words: *Staphylococcus aureus*, MRSA, antibiotic resistance, environment.

Introduction

Staphylococcus aureus is one of the major bacterial agents causing foodborne diseases in humans. This microorganism can cause food poisoning through the production of enterotoxins (Le Loir *et al.,* 2003). Humans are common asymptomatic carriers of enterotoxigenic *S. aureus* in nose, throat, and skin. Thus, food handlers can be an important source of food contamination. The ability to form biofilms allows *S. aureus* to survive in hostile environments such as food industry surfaces (Gotz, 2002)and this enhances the recurrence of food contamination.

The widespread use of antibiotics has provoked an exponential increase in the incidence of antibiotic resistance in several bacterial groups in recent years. Thus, multidrug-resistant *S. aureus* strains are rather common in hospital settings and farms but havebeen also detected in food animals and in food like meat, milk anddairy products, and fishery products (Lee, 2003). With the increased incidence of MRSA, the effectiveness of penicillin and cephalosporins is questioned. In fact many strains of MRSA exhibit resistance to both lactams and

[1] Department of Microbiology, Assam University, Silchar, India.
[2] Department of Life Science and Bioinformatics .Assam University, Silchar, India.
*Corresponding Author: E-mail:sinha_papiya@yahoo.in.
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aminoglycosides (Anupurba et al., 2003). The food chain is considereda potential route of transmission of antibiotic-resistant bacteria to humans.

The present work designed to evaluate the hygienic conditions of food contact surfaces from the dairy and meat sources by screening for the presence of methicillin resistance *S. aureus*. We therefore intend to define the prevalence of MRSA in environmental samples in an effort to provide baseline data for its proper management.

Materials and Methods

Sampling design

A total of 127 samples were collected from different food contact surfaces in dairy and meat sources. This cross sectional study was performed in 14 visits from different semi-urban locations within Cachar district, Assamduring January 2013 to May 2013. Samples were collected aseptically and then transported at 4°C to the laboratory for immediate processing.

Microbiological analysis of the isolates

Selective isolation of the organism was done on Mannitol Salt Agar aseptically and incubated overnight at 37 ^{0}C for 24 h, confirming growth of distinct colonies and then isolates were tested for standard biochemical tests like Gram staining, DNase activity, and coagulase test. The cultures obtained were maintained at -50ºC on Nutrient Broth with 40% glycerol. For each study an overnight culture was inoculated in fresh Nutrient broth (NB) and further incubated to ensure exponential growth conditions.

Antibiogram test

Antibiotic Susceptibility test was performed by Kirby Baeur's disk diffusion Technique (Kirby et al., 2002) in accordance to the protocol recommended by NCCLS (NCCLS 2000) on Mueller-Hinton agar. The plates were incubated at 37 ºC for 24 h to check the zone of inhibition.

A sensitive result obtained was defined as a zone of inhibition that meets the interpretive principles as recommended for inoculation on Mueller Hinton Agar by Standard Method.

The antibiotics discs formulated for the susceptibility test were vancomycin(10µg), oxacillin(5µg),Amikacin(30µg), Gentamicin(20µg), erythromycin (10µg), ceftazidime (30µg), cefuroxime (30µg) ciprofloxacin (30µg), andpenicillin(5 IU).

Figure 1. Antibiotic sensitivity test by disc diffusion method

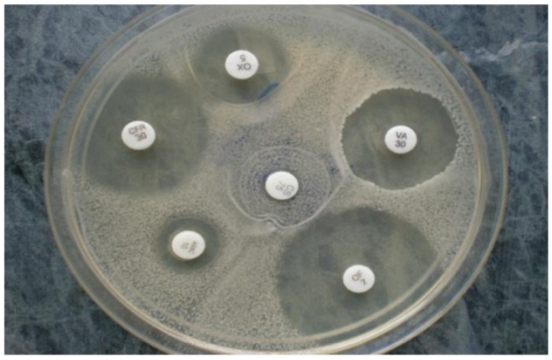


Table 1: Antibiotic susceptibility pattern of *S.aureus* isolates

Antibiotics used	Sn %	Rn%
Vancomycin(10µg)	100%	-
Oxacillin(5µg)	73.33%	26.6%
Amikacin(30µg)	68.33%	31.6%
Ciprofloxacin(30µg)	58%	42%
Gentamicin(20µg)	53%	47%
Ceftazidime(30µg)	51%	49%
Erythromycin(10µg)	46.66%	53.33%
Cefuroxime(30µg)	33.33%	66.66%
Penicillin(5 IU)	13%	87%

* Sn = No. of sensitive isolates, Rn = No.of resistant isolates.

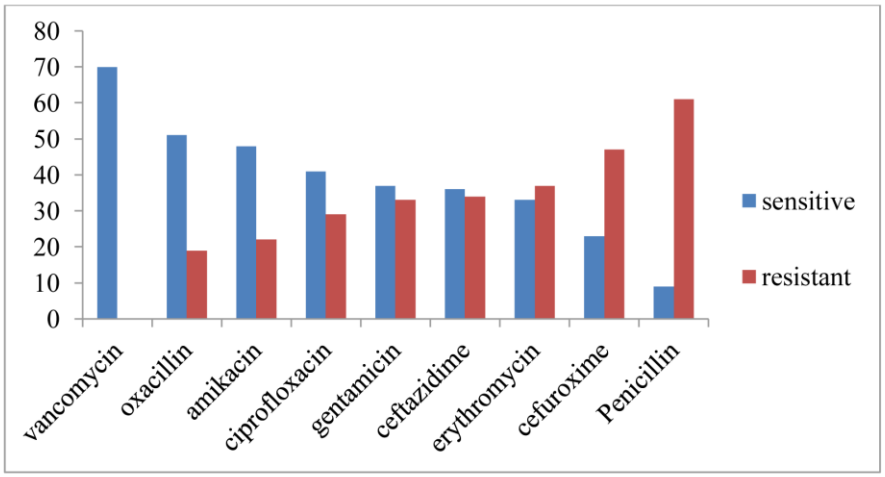
Results

Of the 127 samples screened, 70 (55%) yielded *S. aureus* growth. Twentypercent (20%) of the positive isolates were from meat environments while the rest 35% isolates accounted for food contact surfaces in dairy surroundings. Antimicrobial sensitivity exhibited by isolates are shown in Table 1. Vancomycin exhibited the highest activity (100%). This was followed by oxacillin (73.33%), amikacin (68.33%), ciprofloxacin (58%), gentamicin (53%), ceftadizime (51%), erythromycin (46.66%) and cefuroxime (33.33%). Penicillin recorded least susceptibility, 13%

among the isolates. For Oxacillin, MSSA isolates were noted 73.33% while 26.67% of the isolates were MRSA.

There was a marked difference in the sensitivity pattern of MRSA and MSSA (Figure 2). None of the MRSA isolates was found to be sensitive to penicillin while 9.8% MSSA were sensitive to penicillin. Amongst cephalosporin, ceftazidime showed highest sensitivity 51% MSSA were sensitive while 10.5% MRSA were sensitive to this antibiotic. Sensitivity to macrolide group of antibiotic like erythromycin was seen 41% of MSSA in comparison to15.7% of MRSA.

Figure 2. Graphical representation of sensitivity pattern



Amongst the aminoglycosides, maximum sensitivity was seen in case of amikacin and 60% of MSSA were sensitive while 10.5 % were resistant to the same. All isolates were found to be uniformly sensitive to vancomycin which is the drug of choice for treating infections caused by MRSA.

Discussion

The present study provides information about the incidence of *S. aureus* in the dairy and meat environments (Table 2).

Table 2. Distribution of MRSA and MSSA.

Types of Isolates	Origin of the Isolates	
	Dairy environment	Meat environment
MSSA	23(45%)	28(55%)
MRSA	11(58%)	8(42%)

S. aureus was recovered from 27 out of 442 samples, giving an overall incidence of 6.1%. *S. aureus* had already been detected in a higher proportion (11.7%) from food contact surfaces of meat processing facilities (Gounadaki et al., 2008). Another study (Pala &Sevilla, 2004) recounted a still higher incidence (15.50%) in equipment of a pork dealing out site; however, sampling was not carried out after disinfection of surfaces but, rather, after 5 h of work.

In a study performed in the Czech Republic, *S. aureus* and *S. epidermidis* were isolated from food contact surfaces in dairy and meat processing plants (Schlegelova, 2004). The contamination level of food contact surfaces with *S. aureus* suggests that the handling of livestock as well as cleaning and disinfectionof food industry facilities must be improved.

Our isolates revealed high susceptibility to vancomycin (100%) and this was statistically significant ($p < 0.05$) compared with the other drugs used in the study. This is in agreement with previous studies (Kesha et al., 2003; Shittu& Lin 2006) . However, regular monitoring of the drug's sensitivity is important because resistance has been reported in the USA, Japan and Korea (Shittu& Lin 2006, Classen et al., 2005). Susceptibilities of 72.9 and 71.8% were also noted for ofloxacin and ciprofloxacin respectively (Shittu& Lin, 2006, Saxena et al., 2003) .The high rate of methicillin resistance and that observed with other beta-lactams with our isolates particularly penicillin supports earlier reports(Kesha et al., 2003, O'sullivan & Keane, 2000, Taiwo, 2004, Mi-Na et al., 2002, Liu & Chambers, 2003). Currently, the majority (80%–90%) of *S. aureus* strains in communities are beta-lactamase producers hence resistant to penicillin and ampicillin (Paradisi et al., 2001, Ang et al., 2004).

The distribution of *S. aureus* in the food environment may cause food contamination, which implies some risk of staphylococcal food poisoning in dairy, meat, and fish products, all of which support the growth of *S. aureus. S. aureus* has developed multidrug resistance

worldwide. This is recognized as an environmental hazard to the food supply and to human health, but there are wide variations in incidence as a function of different factors. The presence of methicillin-resistant *S. aureus* (MRSA) is of particular concern. MRSA strains have been found in food-producing animals (Schlegelova, 2004) and different foods (Lee, 2003).

Authors' Note

This manuscript is the authors' original work, has not been published and is not under consideration for publication elsewhere.

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Abstracts published:

- Abstract entitled "Evaluation of sensitivity pattern of MRSA in relation with biofilm production" published in 54th annual conference of Association of Microbiologists of India (AMI-2013).
- Abstract entitled "Evaluation of biofilm production in environmental isolates of coagulase positive Staphylococci in relation with methicillin resistance" published in international conference on Glocal crisis and environmental governance in North-eastern region of India (Nov-2013).
- Abstract entitled "Biofilm as a virulence factor-the cause behind its pathogenicity" published in North-eastern microbiology conference (Apr-2013).
- Abstract entitled "Correlation between biofilm production and multiple drug resistance in clinical and environmental isolates of *S. aureus* of south-Assam" published in a national symposium on Wetland (Nov-2012).

Trainings attended:

- Short term course on statistical methods conducted by BIRU and ISI, Kolkata at Arya vidyapeeth College, Guwahati on 17-22th Nov. 2014.
- Seven days workshop on "Hands on training in molecular biology" at Gauhati university on Apr. 2013.
- Ten days training on "Advance molecular biology technique" on Feb. 2013 at RMRC, Dibrugarh.
- Participated in workshop on Basic Bioinformatics in Dept. of Life Science of Assam University, Silchar on 19-20th Jan. 2009.
- Attended National Workshop on DNA Barcode of life, organized by DBT, Assam University, Silchar on 7th Apr. 2009.
- Attended workshop on Current And Future Research Thrust in life Science in NE-India, conducted by Dept. of Life Science of Assam University, Silchar on 19th Feb. 2008.
- Undergone training workshop on Techniques in Biological Sciences organized under UGC-SAP in Dept. of Life Science of Assam University, Silchar on 25-28th Feb. 2008.