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RESEARCH ARTICLE

EMERGENCE OF MUPIROCIN RESISTANCE IN COMMUNITY ACQUIRED AND HOSPITAL ASSOCIATED STAPHYLOCOCCUS AUREUS

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Abstract

Background
Staphylococcus aureus are ubiquitous gram-positive cocci that have the potential to cause severe diseases. MRSA is an important pathogen, the incidence of which is increasing every year. The remarkable ability of *S. aureus* to develop antibiotic resistance in conjunction with the emergence of highly virulent and/or transmissible strains has established the pathogen as a leading cause of human bacterial infections worldwide. Historically, methicillin-resistant *S. aureus* (MRSA) was found almost exclusively in hospitals and/or health care–related facilities and rapidly became the leading cause of community-associated bacterial infections.

Method
From various clinical samples 120 *S. aureus* strains were isolated by immediately inoculating the samples on Nutrient agar, Blood agar, Mannitol Salt agarplates. Then the culture plates were incubated at 37^{o}C for 24 - 48 hours. After incubation, all isolates were identified by using Gram stain and biochemical methods. Sensitivity tests were performedon Mueller Hinton agar plate by Kirby Bauer's Disc Diffusion Technique and to differentiate between MRSA and MSSA an additional Mupirocin disc were also used.

Result
During the study period (February 2012 to January 2013), a total of 120 *S. aureus* strains were isolated out of which 86 (71.6%) were Hospital acquired compirising 80 MSSA (93.02%) and 06 MRSA (6.97%) isolates,34 (28.33%) were Community acquired *S. aureus*comprising 32 MSSA (94.11%) and 02 MRSA (5.88%) isolates.

Conclusion
The maximum number of *S. aureus*was isolated from pus 50.8% (61 out of 120) and least from ET 2.5% (03 out of 120). In CA-MSSA (32), all strains were 100% sensitive to Ampicillin sulbactum, Linezolid, Cefoxitin, Vancomycin, Oxacillin, Gentamycin, Netillin on the other hand, HA-MSSA (86), all strains were 100% sensitive to Netillin, Gentamycin, Linezolid, Cefoxitin, Vancomycin. In community acquired MRSA strains (02), all are sensitive to mupirocin. The HA-MRSA strains (06), 02 (33%) were sensitive to mupirocin. 01 (16.6%) was high level resistant to mupirocin and 03 (50%) were resistant to low level mupirocin.

Introduction

*Staphylococcus aureus*very commonly causes infections in humans: virtually everyperson will have one or more *Staphylococcus aureus*infections in his or her lifetime.

Most infections occur after an abrasion or cut of the skin due to accidental trauma, like a child that falls on the street. A lesion of the skin, especially when it

has not been cleaned thoroughly, can eventuallybecome painful, red, swollen, and warm, after a day or two. These signs are usually accompanied by a creamy discharge from the wound, known as purulence. This describes the symptoms of an ordinary *S. aureus*wound infection. If such a wound infection occurs, and is cleaned and kept clean, the infection usually subsides and antibiotics are not necessary. One of the reasons that *S. aureus*is a frequent cause of infections is that it cansurvive for months on any type of surface.[1]*S. aureus*cells also possess a wide armamentarium of virulence factors. These virulence factors include factors for adherence, for cell internalization, for evasion of host defense mechanisms, and for invasion of host tissue.[1] With the help of these virulence factors, *S. aureus*is able to colonize the skin and mucous membranes of more than 30% of the human population.[2] It can also colonize the skin and mucous membranes of several animals. This happens on a global scale. Being surrounded or colonized by *S. aureus*is, however, harmless in most cases for a healthy human.

Occasionally such a simple wound infection can become complicated by invasion of the bacteria, where they can cause deep tissue infection and enter the blood stream.[3]Once *S. aureus*cells have entered the blood stream, they will be transported to internal organs, skin and bone, where they can cause new infections, known as metastatic abscesses.[3]This is a serious infection with a high mortality rate, and needs prompt antibiotic treatment.[3]If these infections in healthy humans develop outside the hospital, they are known as community acquired infections. In case these infections develop during hospitalization,they are called nosocomial infections. *S. aureus*ranks second as the cause of nosocomial blood stream infections, that leads to increased morbidity, mortality, hospital stay, and costs.[4-7] Patients admitted to the hospital are, in general, at increased risk for infection.They are ill and, therefore, moderately to severely immune compromised. Hospital treatment usually requires that first line barriers for pathogens, of which the skin is an important one, are intentionally breached, as occurs during surgery or placing of indwelling devices, such as bladder and intravascular catheters. Surgery can result in postoperative wound infections, urinary catheterization in urinary tract infections and intravascular catheters in blood stream infections. Therefore, prevention of these infections is important. Most of these nosocomial *S. aureus*infections are caused by the patient's own flora cells, which were already present on the skin or mucosal membranes prior to hospital admission.[8]

Detection of methicillin-resistant *Staphylococcus aureus*(MRSA) in clinical samplescontinues to be important, since infections due to MRSA have a high morbidity and mortality. Moreover, some MRSA strains have the potential to spread rapidly and colonize other patients. In Netherlands, therefore, patients who are suspected for MRSA carriages are isolated until screening cultures are repetitively negative for MRSA. Methods to detect MRSA in clinical samples should ideally have a high sensitivity and a short time to reporting. To increase the sensitivity one can simply take more screening samples on the same day or on consecutive days, but this is more cumbersome and increases the time to reporting. Another way to increase the sensitivity is to use a broth in addition to agar platesas was demonstrated previously.[1, 5] To increase the sensitivity of the detection of MRSA from a single sample and to improve laboratory efficiency, we developed a new selectivebroth. Subsequently, we compared our routine method of direct plating of specimens ontoblood agar and mannitol salt agar.

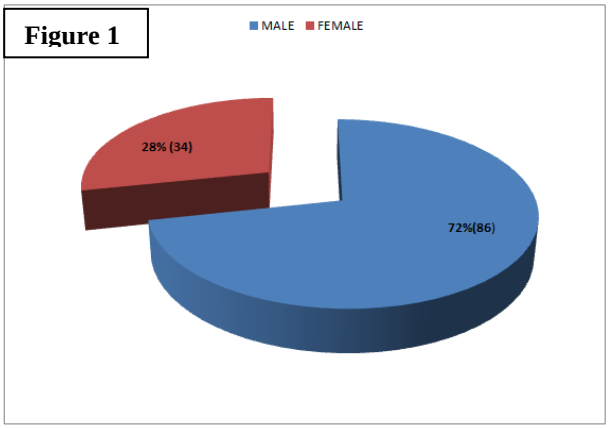
MATERIALS & METHODS

This is a descriptive and prospective study of various clinical samples like pus, blood, urine, sputum,body fluids, catheter tip, throat swab and endotracheal tube over a period of 1 year from February 2012 to January 2013. Total 120 *S. aureus* strains were obtained.from MGM Hospital KamotheNavi Mumbai. All strains were inoculated onto Nutrient agar, Blood agar andMannitol Salt agar media and incubated at 37^{o}C for 24 – 48 hours. After incubation, identification of bacteria cultures was done with standard microbiological technique which included Gram staining and biochemical reactions. The antibiotic sensitivity test of all isolates was performed (according to CLSI guidelines) by modified Kirby Bauer's disc diffusion method on Mueller Hinton agar medium using antibiotic discs of Hi media Laboratories Pvt. Limited, India. An additional Mupirocin disc was also used for sensitivity testing to differentiate between Methicillin Resistant Staphylococcus aureus (MRSA) and Methicillin Sensitive Staphylococcus aureus (MSSA).

Results

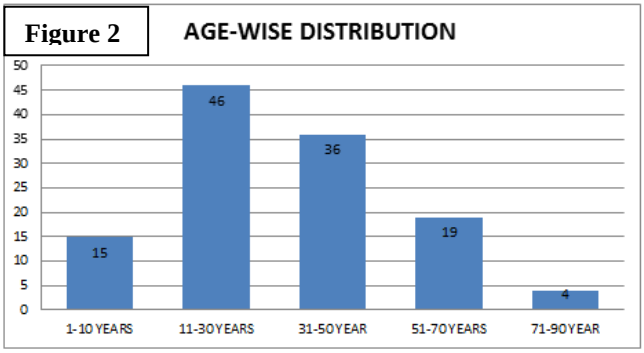
Out of total 120 patients included in this study, 86 (72%) were males and 34) were females (Figure 1).

Figure 1



Surprisingly, highest number of *S. aureus*, 46 (38.33%) were isolated from the age group of 11- 30 years and least in the age group of 71- 90 years (Figure 2).

Figure 2



Out of 120 *S. aureus*isolated, 86 were found to be hospital acquired and 34 werecommunity acquired strains (Figure 3). *S. aureus*with high frequency were isolated from pus 50.8% (61 out of 120) and blood 35% (42 out of 120), followed by urine 8.3% (10 outof 120), throat swab 3.35% (04 out of 120) and ET 2.5% (03 out of 120) (Table 1; Figure 4).

Figure 3

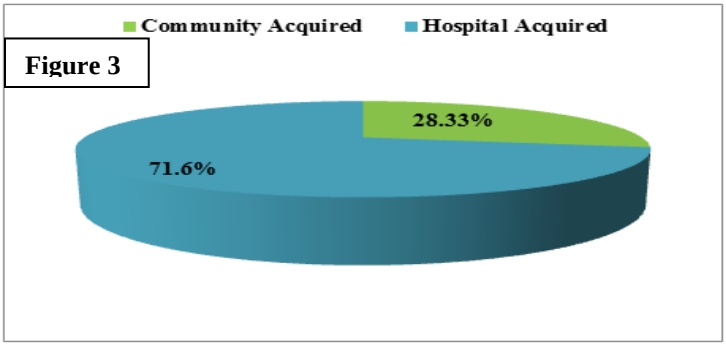
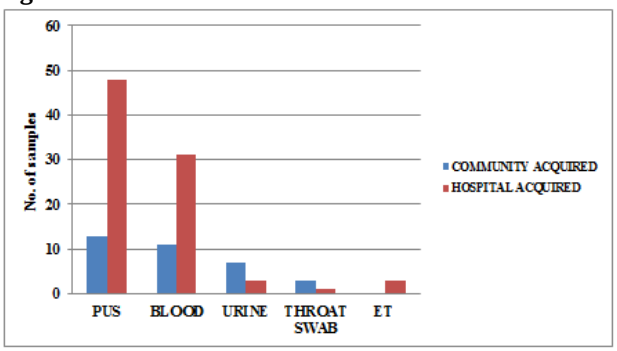


Table 1

Sample	Community Acquired *S. aureus*(N=34)	Hospital Acquired *S. aureus* (N=86)	Total No. (%) (N=120)
Pus	13	48	61 (50.8%)
Blood	11	31	42 (35%)
Urine	07	03	10 (8.3%)
Throat Swab	03	01	04 (3.35%)
Et	00	03	03 (2.5%)

Figure 4.



Out of 34 Community acquired *S. aureus*, 94.11% (32 out of 34) and 5.88% (02out of 34) were MSSA and MRSA respectively (Figure 5). Out of 86 Hospital acquired *S. aureus*, 93.02% (80 out of 86) and 6.97% (06 out of 86) were MSSA and MRSA respectively (Figure 6).

Figure 5

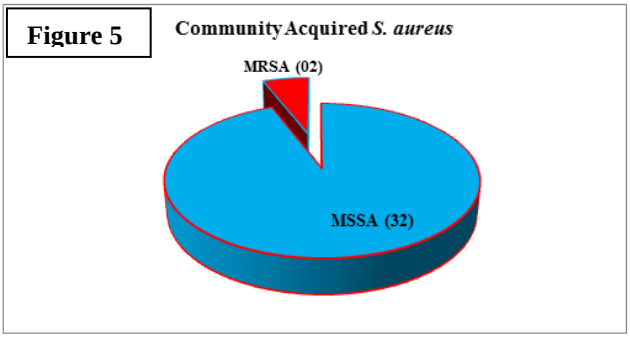
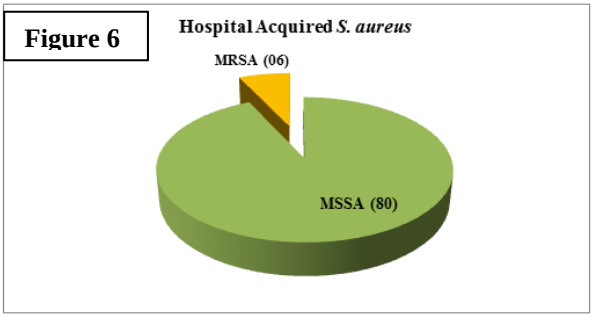


Figure 6



Antibiotic sensitivity pattern of MSSA isolates (Table 2)

Antiboitics	Symbols	Hospital Acquired MSSA strains (n=80) No. (%)	Community Acquired MSSA strains (n=32) No. (%)
Ampicillin sulbactum	AS	76 (95%)	32 (100%)
Cephalexin	CN	75(93.75%)	25 (78.12%)
Cefotaxime	CTX	70 (87.5%)	30 (93.75%)
Levofloxacin	LE	72 (90%)	24 (75%)
Cloxacillin	COX	77(96.25%)	31 (96.87%)
Lincomycin	L	74 (92.5%)	29 (90.62%)
Vancomycin	VA	80 (100%)	32 (100%)
Cephoxitin	CN	80 (100%)	32 (100%)
Co-trimaxazole	COT	70(87.5%)	28 (87.5%)
Tetracycline	TE	76 (95%)	30 (93.75%)
Linezolid	LZ	80 (100%)	32 (100%)
Roxithromycin	RO	60 (75%)	28 (87.5%)
Gentamycin	GEN	80 (100%)	32 (100%)
Netillin	NT	80 (100%)	32 (100%)
Ciprofloxacin	CIP	72 (90%)	27 (84.37%)
Amoxyclav	AMC	72 (90%)	28 (87.5%)
Oxacillin	OX	80 (100%)	32 (100%)

Antibiotic sensitivity pattern of MRSA isolates (Table 3)

Antiboitics	Symbols	Hospital Acquired MRSA strains (n=06) No. (%)	Community Acquired MRSA strains (n=02) No. (%)
Ampicillin sulbactum	AS	02(33.33%)	01 (50%)
Cephalexin	CN	04(66.66%)	01 (50%)
Cefotaxime	CTX	04 (66.66%)	02 (100%)
Levofloxacin	LE	06 (100%)	02 (100%)
Cloxacillin	COX	00 (00%)	00 (00%)
Lincomycin	L	05 (83.33%)	02 (100%)
Vancomycin	VA	06 (100%)	02 (100%)
Cephoxitin	CN	00 (00%)	00 (00%)
Co-trimaxazole	COT	04 (66.66%)	01 (50%)
Tetracycline	TE	05 (83.33%)	02 (100%)
Linezolid	LZ	06 (100%)	02 (100%)
Roxithromycin	RO	04 (66.66%)	02 (100%)
Gentamycin	GEN	05 (83.33)	02 (100%)
Netillin	NT	04 (66.66)	02 (100%)
Ciprofloxacin	CIP	05 (83.33%)	01 (50%)
Amoxyclav	AMC	01 (16.6%)	01 (50%)
Oxacillin	OX	00 (00%)	00 (00%)

Mupirocin resistance in MRSA strains (n=08) (Table 4)

	Hospital acquired MRSA	Community acquired MRSA	Total
Mupirocin (S)	02 (50%)	02 (50%)	04
Mupiricin (R)H	01 (100%)	00 (00%)	01
Mupiricin(R)L	03 (100%)	00 (00%)	03

(S) = Sensitive; (R) H= High level resistence; (R) L=Low level resistence

DISCUSSION

In our study we included 120 S. aureus strains from various clinical samples. Out of these samples 86 (72%) were male and 34 (28%) were female patients. Utajappeet. al., 2008, Heidelberg, Germany, isolated S. aureus out of which 53% were (132 out of 248) from females and 47% were (116 out of 248) from males which is similar to our study.[9] In another study by Jeffrey C. Jones, et. al., 2007, Missouri, S. aureus was isolated from 61% (137 out of 225) Males and 39% (88 out of 225) females.[10]Parraset. al., 1995, Spain, in his study isolated S. aureus from 67% (29 out of 43) males and 33% (14 out of 43) females.[11]

In our study out of 120 patients, 46 (38.33%) patients were in the age group of 11-30 years. In a study by Parraset. al., 1995, Spain, conducted a prospective, open, randomized, comparative study in which they found that maximum number of *S.aureus* infection were in the age group 30-50 years (52.3% i.e. 22 out of 43).[11] Out of 120 *S. aureus* isolated, 86 werefound to be hospital acquired and 34 were community acquired strains. It was seen that rate of MRSA amongst hospital acquired isolates was 6.97% and amongst community acquired isolates was 5.88%.

Our study showed that maximum number of *S. aureus* was isolated from pus 50.8% (61 out of 120) & blood 35% (42 out of 120) , followed by urine 8.3% (10 out of 120), throat swab 3.35% (04 out of 120) and ET 2.5% (03 out of 120). A study by Japoniet. al., 2009, Iran, showed that highest no. of *S. aureus* was isolated from blood 30.6% (109 out of 356), sputum 14% (50 out of 356) & deep wound infection 13.5% (48 out of 356)120. One more similar study by Jamshed Ali Khan et. al, 2008, Pakistan, found that highest no. of *S. aureus* isolates were from pus sample 44% (5,396 out of 12,259).[13] A study by BasudhaShresthaet. al. 2009, Nepal, also supported this result by reporting the highest no. of *S. aureus* isolates 41.31% (351 out of 852) in pus sample.[14]

In our study, out of 120 *S. aureus* strains, 08 were MRSA (6.66 %). Out of 08 MRSA, highest number of isolates i.e. 06 (75%) were from blood. According to study by PoonamSoodLoombaet. al. have shown high prevalence (7.5%) of MRSA colonization (04 MRSA out of 53 samples).[15] A study by Oomenet. al. also supported the result by reporting 2% MRSA colonization[16]

In our study, 02 MRSA were isolated from community and 06 were from hospital setting.In our study, community acquired MSSA (34), all strains were sensitive to Ampicillin sulbactum, Linezolid, Cefoxitin, Vancomycin, Oxacillin, Gentamycin, Netillin 100% (32 out of 32). Whereas, sensitivity to Cloxacillin was 96.87% (31 out of 32), Tetracycline and Cefotaxime 93.75% (30 out of 32).On the other hand, in hospital acquired MSSA (86), all strains were sensitive to Netillin, Gentamycin, Linezolid, Cefoxitin, Vancomycin (100%) (86 out of 86), Ampicillin sulbactum 95% (76 out of 80) and Amoxyclav 90% (72 out of 80). In a study conducted by Nwankwo EOK, et. al., 2010, Nigeria, the percentage sensitivity for the MSSA were recorded as follows; Methicillin (100%), Ciprofloxacin (68%), Ofloxacin (93%), Amoxycillin/clavulanic acid (62%), Ceftriaxone (90%), Gentamicin (75%) while Ceftazidime was (92%). All the isolates were sensitive to Vancomycin.[17]

In our study, community acquired MRSA (02), all strains were sensitive to Cefotaxime, Gentamycin, Levofloxacin, Lincomycin, Linezolid, Vancomycin and Netillin 100% (02 out of 02) and Ciprofloxacin 50% (01 out of 02), whereas, none of them were sensitive to Cloxacillin. In a study by Nwankwo EOK, et. al., 2010, Nigeria, the antibiotic sensitivity profile of MRSA to various antibiotics was as follows, Ciprofloxacin (64%), Ofloxacin(90%), Amoxycillin/clavulanic acid (31.7%), Ceftriaxone (75%), Gentamicin (18%) and Ceftazidime (79%)[17]

In community acquired MRSA (02), all were sensitive to 5µg mupirocin. The hospital acquired MRSA (06), 02 (33%) were sensitive to mupirocin. 01 (16.6%) was high level resistant to mupirocin and 03 (50%) were low level resistant to mupirocin. In a study by Naira Elane Moreira de Oliveira, et. al., 2007, Brazil, out of 124 *S. aureus*strains tested for mupirocin sensitivity pattern, they found 68% (85 out of 124) were sensitive to mupirocin, 16.12% (20 out of 124) and 15.32% (19 out of 124) were resistant to high level and low level mupirocin respectively.[18]

CONCLUSION

Methicillin-resistant *S.aureus* infections are no longer confined to health care facilities or individuals who have risk factors for infection. Although MRSA infections are those affecting skin and soft tissue,there is potential for serious, life-threatening disease. Therefore, accurate and timely diagnosis of MRSA infections is a crucial step toward successful treatment. Last but not least, good personal hygiene, such as covering wounds, washing hands, no sharing of personal items, and maintaining a clean environment, is key in preventing MRSA infections.Indiscriminate use of antibiotics has lead to the development of antibioticresistant strains for commonly used antibiotics such as quinolones and cephalosporins. Thus, a detail study is required with regard to proper antibiotic usage, susceptibility testing irrespective of the organisms isolated from samples to find out the resistance patterns.

Hence, we believe that our study will provide information to Clinicians inprescribing effective and appropriate antimicrobial agents against infections and will help to improve health care system.

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