BACTERIOLOGICAL STUDY OF COAGULASE-POSITIVE AND COAGULASE-NEGATIVE STAPHYLOCOCCI IN RELATION TO METHICILLIN SENSITIVITY TESTING

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ABSTRACT

BACKGROUND

Staphylococcus aureus has long been recognised as an important pathogen in human disease. Staphylococci infection occurs regularly in hospitalised patients and has serious consequences despite antibiotic therapy. Shortly after introduction of methicillin after clinical use Methicillin-Resistant Staphylococcus Aureus (MRSA) were identified in many countries and become one of the most common causes of nosocomial infections.

The aim of the study is to know the methicillin sensitivity of both coagulase-negative and coagulase-positive staphylococci isolated from various samples.

MATERIALS AND METHODS

100 strains of staphylococci both coagulase positive and coagulase negative were isolated in the Department of Microbiology from various clinical samples. They were confirmed by morphology, staining methods and by using standard bacteriological procedures and biochemical reactions. Antibiotic susceptibility testing was performed by Kirby Bauer disc diffusion test.

RESULTS

Predominant species from pus were S. epidermidis (42.42%) and from sputum S. haemolyticus (31.81%) from blood S. haemolyticus (53.33%). 53% of strains produced beta-lactamase. Majority 47.22% by S. epidermidis from pus followed by S. haemolyticus 23.33% from pus. Beta-lactamase production was least from throat swab (5.55%). Out of 32 coagulase-positive staphylococci tested to methicillin 15 (46.87%) were found to be sensitive, 17 (53.13%) were found to be resistant. Out of 68 coagulase-negative staphylococci tested, 13 (19.11%) were found to sensitive and 55 (80.88%) were found to be resistant. 72% of strains were sensitive to novobiocin and 28% resistant to novobiocin. 43% showed drug resistance to 2 drugs. 14% to 3 drugs and 5 drugs. 6% of staphylococci sensitive to all the 10 drugs.

CONCLUSION

MRSA is a type of bacteria that is resistant to a number of widely used antibiotics. This means MRSA infections can be more difficult to treat than other bacterial infections. In recent years, rates of MRSA have fallen because of increased awareness of the infection by both medical staff and the public. However, MRSA still places a considerable strain on healthcare services. Some people who need to be admitted to hospital will have MRSA screening beforehand, but there are also some things you can do yourself to reduce your risk of becoming infected. These include:

- Washing your hands frequently - especially after using the toilet and before and after eating.
- Following any advice you are given about wound care and devices that could lead to infection (such as urinary catheters).
- Reporting any unclean toilet or bathroom facilities to staff – don't be afraid to talk to staff if you're concerned about hygiene.

If you're visiting someone in hospital, you can reduce the chance of spreading MRSA by cleaning your hands before and after entering the ward. You should also use hand wipes or hand gel before touching the person you're visiting.

KEYWORDS

Staphylococcus Aureus, Methicillin Sensitive, Methicillin Resistance.

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BACKGROUND

Staphylococcus aureus has long been recognised as an important pathogen in human disease. Staphylococci infection occurs regularly in hospitalised patients and has serious consequences despite antibiotic therapy. Shortly after introduction of methicillin after clinical use Methicillin-Resistant Staphylococcus Aureus (MRSA) were identified in many countries and become one of the most common causes of nosocomial infections.[1,2]

Staphylococcus aureus was major cause of hospital-acquired infections causing high morbidity and mortality throughout the world. The proportion of Methicillin-Resistant

Staphylococcus Aureus (MRSA) has raised worldwide during the last two decades. The rapidity with which methicillin resistant developed created therapeutic problems for physicians, management difficulty for nursing and confusion for infection, control practitioners and resource allocation uncertainties for hospital administrators.[3]

Staphylococci are the important hospital pathogen and infections are common with Methicillin-Resistant Staphylococcus Aures (MRSA) despite antibiotic therapy among hospitalised patients in whom medical, surgical therapy provides easy and related to foreign bodies such as intravascular and peritoneal dialysis equipment catheters, prosthetic joints and heart valves.[4]

As a group, staphylococci are also among the most frequently isolated bacteria in our laboratory. The major problem facing the laboratory is to distinguish between clinically significant pathogenic strains of staphylococci from others. Majority of infections assumed to be hospital strains. Recent reports on surveillance data taken from the "National Nosocomial Infection Surveillance" system from latter half of 1990's and 2000 onwards have indicated that coagulase-negative Staphylococci are among the 5 most commonly reported pathogens in hospitals when compared to coagulase-positive staphylococci.[5]

Repeated isolated of the organism from a patient with same biochemical reactions suggests that the organism has originated from the single clone and if the same organism with all typical characters isolated from another patient signifies that it is a hospital strain. Hervert et al (1998) proposed a biotyping system that is staphylococci isolates. Baird-Parker (1960) proposed classifying staphylococci into six groups and micrococci into seven groups based on characters like coagulase, phosphatase, aerobic and anaerobic acid production from mannitol, lactose, maltose, acetoin production and grown at 10^0C.[6]

Increasing incidence and importance of coagulase-negative staphylococci infections in number has probably resultant from indiscriminate use of antibiotics and corticosteroids in therapy. Importance of early diagnosis should be stressed, if not treated properly in time may lead to various complications as they are present in dialysis equipment, catheters, transplant equipment and early colonising on skin and mucous membranes in premature infants.[7,8]

MATERIALS AND METHODS

100 strains of staphylococci both coagulase positive and coagulase negative were isolated in the Department of Microbiology from various clinical samples. These include from different sources of pus, urine, sputum, blood, throat swabs, cerebrospinal fluid, vaginal swab, corneal scraping and formed the material for the present study.

The strains selected were showing predominant growth in primary culture and also in mixtures. They were confirmed by morphology, staining methods and other tests like slide coagulase and confirmed with tube coagulase with rabbit plasma or sheep plasma. The following tests were performed to demonstrate their characters.

- Tests for production of enzymes- oxidase, catalase.
- Tests for utilisation of carbohydrates (i.e., fermentation of sucrose, maltose, xylose, mannitol and lactose).
- Beta-lactamase production test by iodometric method.
- Slime production by use of congo red agar medium.
- Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion technique. Various antibiotics tested were methicillin, erythromycin, kanamycin, gentamicin, ampicillin and ofloxacin.

RESULTS

Total No. Processed	Coagulase-Positive Staphylococci No.	%	Coagulase-Negative Staphylococci No	%
100	32	32%	68	68%

Table 1

Table No. 1 shows out of 100 isolates tested 32 (32%) were found to be coagulase-positive staphylococci and 68 (68%) were found to be coagulase-negative staphylococci. The Table No. 1 shows the predominant are coagulase-negative staphylococci, i.e. (68%).

Male		Female	
Number	**%**	**Number**	**%**
54	54%	46	46%

Table 2. Sex Wise Distribution of Staphylococci

Table No. 2 shows sex wise distribution of staphylococci out of 100 isolated strains males form the major group 54 (54%) followed by females 46 (46%).

Sl. No.	Age in Years	Number	%
1.	0-10	Nil	Nil
2.	11-20	33	33%
3.	21-30	17	17%
4.	31-40	16	16%
5.	41-50	17	17%

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6.	51-60	6	6%
7.	61-70	9	9%
8.	71-80	2	2%
	Total	**100**	**100%**

Table 3. Age Wise Distribution of Staphylococci

Table No. 3 shows age wise distribution of staphylococci the youngest age group 11 years 1 male and 1 female and oldest age is 80 years 2 females. The following is the age wise distribution as follows 0 to 10 years nil cases, 11 to 20 years 33 (33%), 21 to 30 years 17 (17%), 31 to 40 years 16 (16%) 41 to 50 years 17 (17%), 51 to 60 years 6 (6%), 61 to 70 years 9 (9%), 71 to 80 years 2 (2%).

Sl. No.	Name of Specimen	Number
1.	Pus	33
2.	Urine	23
3.	Sputum	22
4.	Blood	15
5.	Throat swab	3
6.	Cerebrospinal fluid	2
7.	Vaginal swab	1
8.	Corneal scraping	1
	Total	**100**

Table 4. Specimen wise Distribution of Coagulase-Positive Staphylococci and Coagulase-Negative Staphylococci

Table No. 4 shows specimen wise distribution of coagulase-positive and coagulase-negative staphylococci.

Out of 100 isolates, majority of the isolates from the pus 33% followed by urine 23% remaining from the sputum 22%, blood 15%, throat swap 3%, cerebrospinal fluid 2%, vaginal swap 1%, corneal scraps 1%. Again the distribution of coagulase-positive and coagulase-negative staphylococci from different samples as follows pus coagulase positive 7 (21.21%), coagulase negative 26 (78.88%), urine coagulase positive 7 (30.43%), coagulase negative 16 (69.56%), sputum 10 (45.45%), coagulase negative 12 (54.55%), blood coagulase positive 2 (66.66%), coagulase negative 33.33%, cerebrospinal fluid coagulase positive 2 (100%), corneal scraping coagulase-negative staphylococci 1 (100%).

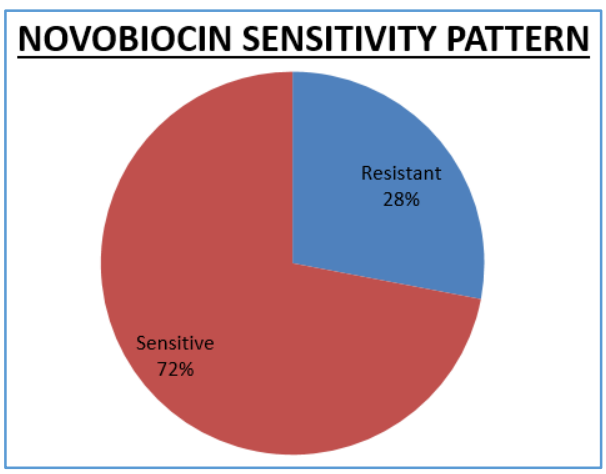

Table 5

Sl. No.	Species	Total No	Blood		Pus		Urine		Sputum Swab		Throat		CSF		Vaginal Swab		Corneal Scraping	
			R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
1.	S. Epidermidis	36	-	-	-	22 (61.11%)	-	-	-	11 (30.5%)	-	2 (5.55%)	-	-	-	1 (2.77%)	-	-
2.	S. Haemolyticus	30	-	13 (43.33%)	-	9 (30%)	-	2 (6.66%)	-	4 (13.33%)	-	1 (3.33%)	-	1 (3.33%)	-	-	-	-
3.	S. Saprophyticus	18	-	-	-	-	16 (88.8%)	-	-	1 (5.55%)	-	-	-	-	-	-	-	1 (5.55%)
4.	S. Cohnii	10	-	-	2 (20%)	-	4 (40%)	-	4 (40%)	-	-	-	-	-	-	-	-	-
5.	S. Hominis	2	-	-	-	-	-	-	-	1 (50%)	-	-	-	1 (50%)	-	-	-	-
6.	S. Xylosus	2	-	-	-	-	1 (50%)	-	1 (50%)	-	-	-	-	-	-	-	-	-
7.	S. Warneri	2	-	2 (100%)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8.	S. Capitis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9.	S. Simulans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Total	**100**	**-**	**15**	**2**	**31**	**21**	**2**	**5**	**17**	**-**	**3**	**-**	**2**	**-**	**1**	**-**	**1**

Table 6. Showing Strains Sensitive to Novobiocin Species Wise and Specimen Wise

Table No. 5 and 6 shows total number of sensitivity pattern of novobiocin species wise and specimen wise. Total number of staphylococci were tested 100. Among them, 72 (72%) were sensitive. 28 were novobiocin resistant. Specimen wise, maximum number of staphylococci, 22 out of 36 (61.1%) were found in pus and maximum number of staphylococci 16 out of 18 (88.88%) resistant to novobiocin.

Species wise, predominant species sensitive to novobiocin were S. epidermidis form pus 22 out of 36 (61.1%) and the predominant resistant species S. saprophyticus 16 out of 18 (88.8%) from urine.

Sl. No.	Species	Tot. No.	Blood		Pus		Urine		Sputum		Throat Swab		CSF		Vaginal Swab		Corneal Swab	
			+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
1.	S. epidermidis	36	-	-	17	6	-	-	5	6	2	-	-	-	-	-	-	-
			-	-	47.22%	16.66%	-	-	13.88%	13.88%	5.55%	-	-	-	-	-	-	-
2.	S. haemolyticus	30	6	8	7	2	-	2	2	2	-	1	-	-	-	-	-	-
			16.66%	26.66%	23.33%	6.66%		6.66%	6.66%	6.66%	-	-	-	-	-	-	-	-
3.	S. saprophyticus	18	-	-	-	-	10	7	1	-	-	-	-	-	-	-	-	-
			-	-	-	-	55.55%	38.88%	5.55%	-	-	-	-	-	-	-	-	-
4.	S. cohnii	10	-	-	-	-	-	4	-	3	-	-	1	1	-	-	-	1
			-	-	-	-	-	40%	-	30%	-	-	10%	10%	-	-	-	10%
5.	S. hominis	2	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-
			-	-	50%	-	-	-	-	50%	-	-	-	-	-	-	-	-
6.	S. xylosus	2	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	100%	-	-	-	-	-	-	-	-
7.	S. warneri	2	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-
			-	50%	-	-	-	-	-	-	-	-	-	-	50%	-	-	-
8.	S. capitis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9.	S. simulans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Total	**100**	**6**	**9**	**25**	**8**	**10**	**13**	**8**	**14**	**2**	**1**	**1**	**1**	**1**	**-**	**-**	**1**

Table 7. Specimen and Species that Produce Beta-Lactamase

Table No. 7 shows beta-lactamase production specimen wise and species wise. S. epidermidis from pus were the major species producing beta-lactamase were 17 out of 23 (73.91%). The next major species of staphylococci producing beta-lactamase were S. saprophyticus from urine where 10 out of 17 (58.82%) produced beta-lactamase.

Coagulase-Positive Staphylococci			Coagulase-Negative Staphylococci		
No. Tested	**Sensitivity**	**Resistant**	**No. Tested**	**Sensitivity**	**Resistant**
32	15 (46.87%)	17 (53.13%)	68	13 (19.12%)	55 (80.88%)

Table 8. Methicillin-Sensitivity Pattern of Staphylococci

Table No. 8 shows methicillin-sensitivity pattern of staphylococci. Out of 32 coagulase-positive staphylococci tested, 15 (46.87%) were found to be sensitive 17 (53.13%) were found to be resistance. Out of 68 coagulase-negative staphylococci tested, 13 (19.1%) were found to be sensitive and 55 (80.88%) were found to be resistant.

Sl. No.	Drug Resistance	Number	Percentage (%)
1.	Resistance to 1 Drug	13	13
2.	Resistance to 2 Drug	43	43
3.	Resistance to 3 Drug	14	14
4.	Resistance to 4 Drug	7	7
5.	Resistance to 5 Drug	14	14
6.	Resistance to 6 Drug	2	2
7.	Resistance to 7 Drug	1	1
8.	Sensitive to all 10 Drugs	6	6
	Total	**100**	**100%**

Table 9. Incidence of Drug Resistance - 100

Table No. 9 shows incidence of drug resistance in coagulase-positive staphylococci and coagulase-negative staphylococci. Total number of drugs tested was 10. They were penicillin, ampicillin, methicillin, novobiocin, ofloxacin, cephalexin, gentamicin, erythromycin, kanamycin and co-trimoxazole. Total number of isolates tested was 100. Maximum number was 43 out of 100 showed resistances to two drugs 43%, 14 (14%) were resistance to three drugs. The next in the order of number is again 14 (14%) were resistance to 5 drugs, 13 (13%) to 1 drugs. Staphylococci (coagulase positive) and (coagulase negative) sensitive to all drugs were 6 (6%).

Sl. No.	Antibiotic	S. epidermidis	S. haemolyticus	S. saprophyticus	S. cohnii	S. hominis	S. xylosus	S. warneri	S. capitis	S. simulans
1	Penicillin	22 (61.1%)	18 (60%)	17 (94.4%)	4 (40%)	2 (100%)	2 (100%)	2 (100%)	-	-
2	Ampicillin	14 (38.8%)	13 (43.33%)	14 (77.7%)	5 (50%)	2 (100%)	1 (50%)	1 (50%)	-	-
3	Methicillin	26 (72.22%)	16 (53.33%)	8 (44.44%)	5 (50%)	1 (50%)	-	-	-	-
4	Novobiocin	-	-	18 (100%)	10 (100%)	2 (100%)	-	-	-	-
5	Ofloxacin	14 (38.8%)	10 (33.33%)	6 (33.33%)	6 (60%)	2 (100%)	1 (50%)	1 (50%)	-	-
6	Cephalexin	16 (44.44%)	12 (40%)	12 (66.66%)	6 (60%)	-	2 (100%)	-	-	-
7	Gentamicin	18 (50%)	8 (26.66%)	3 (16.66%)	3 (30%)	-	-	-	-	-
8	Erythromycin	12 (33.33%)	11 (36.66%)	7 (38.88%)	4 (40%)	2 (100%)	-	-	-	-
9	Kanamycin	6 (16.66%)	8 (26.66%)	6 (33.33%)	2 (20%)	2 (80%)	-	-	-	-
10	Co-trimoxazole	24 (66.66%)	18 (60%)	14 (77.77%)	8 (80%)	2 (100%)	-	2 (100%)	-	-
	Total	**36**	**30**	**18**	**10**	**2**	**2**	**2**	-	-

Table 10. Showing Antibiotic Resistance in Relation to Species

Table No. 10 shows antibiotic resistance in relation to species. Total number of drugs used were 10. They were penicillin, ampicillin, methicillin, novobiocin, ofloxacin, cephalexin, gentamicin, erythromycin, kanamycin, co-trimoxazole. Total number of isolated tested were 100. Maximum, i.e. 36 out of 100 (36%) highest were S. epidermidis 36% were resistant to methicillin, 72.22% followed by co-trimoxazole, 66.66% the other species name, S. saprophyticus showing resistance to novobiocin 18 (100%) followed antibiotic resistance in relation to species is S. haemolyticus showing resistance to the antibiotics tested as follows penicillin 60%, co-trimoxazole 60% and methicillin 53.33%.

DISCUSSION

One hundred strains of staphylococci isolated from various clinical samples were analysed. The results were discussed below in detail with others study. In the present study, majority of the strains isolated were from pus 33 (33%), the next majority were from sputum 22 (22%), the remaining were from blood 15 (15%), throat swab 3 (3%), cerebrospinal fluid 2 (2%), vaginal swab 1 (1%) and corneal scraping 1 (1%).

Assadullah et al July 2003 reported 34.25% of staphylococci from pus samples. The next majority were from urine samples 20% were noticed from the same above study coinciding with our study.

Rajadurainpandi et al 2006 in their study on prevalence and antimicrobial studied of Methicillin-Resistant Staphylococcus Aureus (MRSA) found 33.6% of Methicillin-Resistant Staphylococcus Aureus (MRSA) from pus samples in their study. The present study majority of the strains isolated were from pus 33 (33%) coinciding with the findings of the above reference.

Wilson and Stuart in 1965 reported 4.4% coagulase-negative staphylococci. Pulverer and Pilbich in 1971 studied 256 cases and observed 50% were due to coagulase-negative staphylococci in pyogenic infections. Jack et al in 1980 in their study reported only one case of intra-abdominal abscess and isolated S. epidermidis. Claudio F-Lanta et al in 1985 and Leroy F. Harris in 1985 in different studies reported a small number of isolated from intra-abdominal abscess (3 and 2 cases, respectively). S. Rajeswara Rao in 1993 reported 38.5% isolation of coagulase-negative staphylococci from pus and wound swabs. Jayanthi Phatak et al in 1994 reported maximum incidence (21.6%) of coagulase-negative staphylococci in wound infections. In the present study, coagulase-negative staphylococci 26 out of 33 (78.78%) pus samples are predominant from different samples than coagulase-positive staphylococci 7 out of 33 (21.21%) of pus samples.

In the present study, 23 (23%) staphylococci isolated from urine samples and the predominant species S. saprophyticus 9 out of 23 (39.13%) of urine samples. G. Wallrak et al in 1978 who studied 661 urinary specimens out of which 173 were S. saprophyticus. Peggy et al in 1980 studied 68 strains from urinary samples. From there, 64 were identified as S. saprophyticus, 3 as S. epidermidis and one as micrococcus.

In our study, the incidence of S. saprophyticus from the urine samples predominant.

The present study correlated well with the studies above references. The present study correlated well with the studies of G. Wallrak et al (1978) and Peggy et al (1980) and the species isolated were the same.

Comparative Studies

Authors	Year of Isolation	No. of Isolates	Major Species
Peggy et al	1980	60	S. saprophyticus 4 (94.1%)
Robert H. Lantham et al	1983	43	S. saprophyticus 27 (62.7%)
P. Bhalla and Agarwal	1986	148	S. saprophyticus 9 (39.8%)
Peggy et al	1980	60	S. saprophyticus 4 (94.1%)
Robert H. Latham et al	1983	43	S. saprophyticus 27 (62.7)
P. Bhalla and Agarwal	1986	148	S. saprophyticus 9 (39.8%)
Present Study	2006	23	S. saprophyticus 9 (39.13)
Otakar et al	1980	5	S. epidermidis 5 (100%)
Timothy E. West et al	1986	35	S. epidermidis 29 (82.8%)
Loreen A et al	1990	108	S. epidermidis 103 (95.3%)
Present study	2006	68	S. epidermidis 27 (39.70%)
V. M. Mahajan	1979	Eye discharge	S. epidermidis (subgroup 2)
N. Vijayalakshmi et al	1980	Various Clinical Samples	S. epidermidis
P. Bhalla and D. S. Agarwal	1986	Urine	S. saprophyticus
Pushpa Agarwal	1991	Various Clinical Samples	S. epidermidis
S. Rajeswara Rao	1993	Various Clinical Samples	S. haemolyticus
Jayanti Phatak	1994	Urine	S. epidermidis
Present Study	2015	Various Clinical Samples	S. epidermidis

CONCLUSION

MRSA is a type of bacteria that is resistant to a number of widely used antibiotics. This means MRSA infections can be more difficult to treat than other bacterial infections. In recent years, rates of MRSA have fallen because of increased awareness of the infection by both medical staff and the public. However, MRSA still places a considerable strain on healthcare services. Some people who need to be admitted to hospital will have MRSA screening beforehand, but there are also some things you can do yourself to reduce your risk of becoming infected. These include:

- Washing your hands frequently - especially after using the toilet and before and after eating.
- Following any advice you are given about wound care and devices that could lead to infection (such as urinary catheters).
- Reporting any unclean toilet or bathroom facilities to staff – don't be afraid to talk to staff if you're concerned about hygiene.

If you're visiting someone in hospital, you can reduce the chance of spreading MRSA by cleaning your hands before and after entering the ward. You should also use hand wipes or hand gel before touching the person you're visiting.

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