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Research Article

Antimicrobial Susceptibility Patterns of Gram Positive Isolates from Saudi Arabian Hospital

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Abstract

The study was carried out in Jazan general hospital of Saudi Arabia. The main focus lies in recovery of *Staphylococcus aureus* isolates from different places confined to the hospital to determine the chances of nosocomial infection and its susceptibility pattern against selected antibiotics. Total 332 cfu were observed at five different places viz. OPD, Emergency, Ward, Corridor, and thumb impression of ward attendants & technical staff. No cfu was observed in Operation Theater. Out of total 332 only 63 cfu were found typical *of gram positive Staphylococcus aureus,* which were analyzed for its phenotypic characteristic and antimicrobial susceptibility. The biochemical characterization was done by API 20E and five different biochemical patterns (I-V) were detected among isolates. They were found distributed in almost all the areas; however the most predominant of all i.e. biochemical patterns I was found in each area of the hospital. The isolates vary in its antibiotic susceptibility pattern. All isolates (100%) were found to be susceptible to Gatifloxacin, Ampicillin sulbactam, Ciprofloxacin and Sulphamethoxazole trimethoprime. Chloramphenicol and Cefotaxime sodium shows 95%, Ofloxacin 96%, Tetracycline 95%, Vancomycin, and Methicillin 93%, Cefactor 85%, Cefeprime 84%, Gentamycin 82%, Latmoxef 77% and Cefprozoil & Fusidic acid 74% susceptibility. However all isolates were found to be resistant to Bacitracin, Aztreonam, Azithromycin and Cefixime.

Keywords: *Staphylococcus aureus*; UTI; Antimicrobial susceptibility; CFU (Colony forming units); OPD (Outdoor patient department)

Introduction

The *Staphylococcus aureus* causes acute suppurative inflammation being the most virulent forms of septicemia and pyaemia [1]. More than 100 years later, before the role of *Staphylococcus aureus* was established in sepsis and abscess formation it still remain a versatile and dangerous pathogen in human [2]. The frequency of both community acquired and hospital acquired (nosocomial) Staphylococcal infections have increased steadily, with little change in overall mortality (CDC, 1998). The data from the national nosocomial infections surveillance (NNIS) system from 1986 to 1990 reported *E. coli* (13.7%), *Staphylococcus aureus* (11.2%), *Enterococci* (10.7%), and *Pseudomonas aeruginosa* (10.1%). These data show that *Staphylococcus aureus* remain important nosocomial pathogen and that the distributions of pathogens differ by site and hospital location [3].

Jazan being the new developing province of Saudi Arabia and the government general hospital being developed recently, no such survey for nosocomial pathogens was carried out before. In the present study therefore Jazan general hospital was selected were patients from surrounding area are admitted. Isolation of *Staphylococcus aureus* causing nosocomial infection was first of its kind in the hospital and the whole Gizan province in general. The main purpose of this study was to survey different biochemical types of *Staphylococcus aureus* and its susceptibility to antimicrobial agents, so as to provide data that may help in selecting the drug of choice and treatment of nosocomial infection. Also it may help hospital authorities to improve upon the existing conditions to reduce the chances of nosocomial infection.

Review of literature

The susceptibility of bacteria against the antibiotics is an emerging problem and lot of studies has been carried out in this direction. A study was carried out by [4], on nosocomial blood stream infections by gram negative organism from SENTRY hospitals in Canada, USA and latin America. According to their findings *E.coli* was the most prominent isolate followed by *Klebsiella sps*, *Pseudomonas aeruginosa* and *Enterobacter* species. The effective antibiotics against these bacteria were Levofloxacin, Ciprofloxacin, Gatifloxacin and Trovafloxacin. However resistant phenotypes of *E. coli* and *Klebsiella* species against ESBL were reported from Latin America.

In another study carried out by [5], the gram positive isolates from European medical centers were tested for their susceptibility pattern against different antibiotics. Among these were *Staphylococcus aureus, Enterococcus faecium*, beta haemolytic *Streptococci,* and *viridans group Streptococci*. Methicillin resistance rates were 26.7% for *Staphylococcus aureus*. The rates of MRSA varied for different countries, viz: 0.6% in Sweden to 40.2 – 43.0% in Belgium, Grrece, Ireland, UK and Israel. However, more than 99.9% isolates were found to be susceptible to Daptomycin according to United States Food and Drugs Administration.

In the present study we have concentrated on isolation of gram positive isolates mainly *Staphylococcus aureus* causing nosocomial infections from Jazan general hospital and its antibiotic susceptibility pattern.

Materials and Methods

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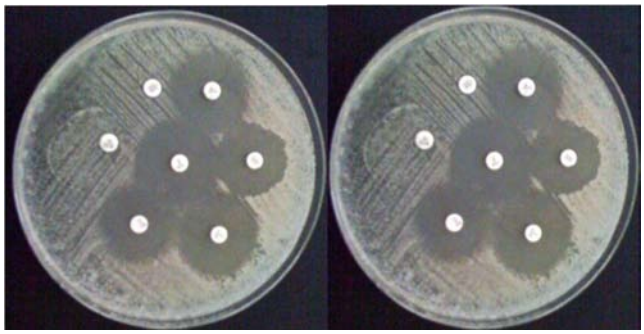
Table 1: Distribution of total bacterial cfu count and cfu typical *of' Staphylococcus aureus* with its percentage, recovered from different places in Jazan region hospital, Jazan, KSA.

S.No	Place	Total bacteria.cfu count (average)	Total cfu typical of *S. aureus* (average)	% of cfu typical of *S. aureus*
1	Outdoor patient Department	132	30	23
2	Emergency ward	82	15	18
3	Patient's ward	11	02	18
4	Corridor / Passage	23	04	17
5	Thumb impression of ward boys	84	12	14
6	Operation Theatre	Nil	Nil	Nil
	Total	332	63	
	Mean	55.33	10.5	
	Standard deviation	49.52	10.226	



Picture 1: Antibiotic susceptibility / resistant pattern of *S.aureus* isolates.

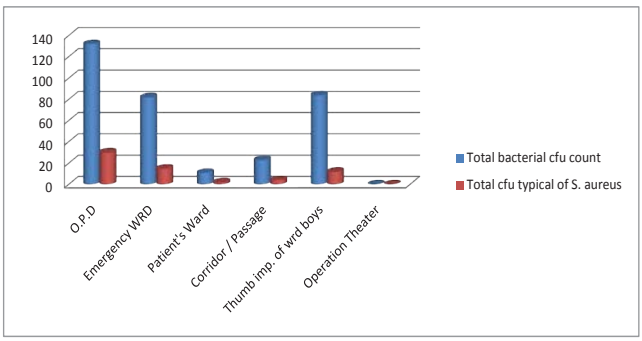


Figure 1:

Sampling

The permission was sought by the medical director Jazan general hospital for the survey and sampling within the premises of the hospital. The places from where the sampling was done were O.P.D, Emergency ward, Patient's ward, Corridor (Passage), O.T and Thumb impression of ward boys working in various departments. The sterilized Petri plates containing mannitol salt agar specific for *Staphylococcus aureus* were exposed for 30 minutes at above mentioned places. The thumb impressions of ward boys were taken on the surface of the agar. The experiment was repeated in the hospital for three times exposing new plates of mannitol salt agar (MSA) on different days in a week. The plates were than incubated at 37°c for 24 hours in laboratory.

Phenotypic characterization of staphylococcus aureus

The isolates of *Staphylococcus aureus* were further identified on the basis of its morphology including gram stain and biochemical characterization using API -20E test kit (bioMerieux, Marcy-l'Etoile, France). After incubation, the color reactions are read and are matched with the standard chart of the known *Staphylococcus aureus* ATCC 29213 /NCTC 12973. The Staphylococcus aureus was also confirmed by Vitek.

Susceptibility to selected antibiotics

The antimicrobial susceptibility of the *Staphylococcus aureus* isolates was determined by disk diffusion method of Kirby-Bauer technique according to the guidelines of the national committee for clinical laboratory standards (NCCLS). The test was done in triplicate and a control was run simultaneously using *Staphylococcus aureus* ATCC 29213. The antibiotics tested were Bacitracin (0.05units), Gatifloxacin (5 ug), Tetracycline (30 ug), Cefotaxime sodium(30 ug), Ampicillin sulbactum (20 ug), Ciprofloxacin (5 ug), Aztreonam (30 ug), ofloxacin (5 ug), Cofactor (30 ug), Azithromycin (15 ug), Sulphamethoxazole trimethroprime (25 ug), Ceprozoil (30 ug), Latamoxef (30 ug), Cefixime (5 ug), Gentamycin (10 ug), Vancomycin (30 ug), Fusidic acid (30 jag), Chloramphenicol (30 ug), Methicillin (5 ug), and Cefeprime (30 ug).

Result and Discussion

Sampling

The plates containing mannitol salt agar exposed to different places in the Jazan general hospital showed varied results in Colony Forming Units (cfu) of bacterial population. The colonies typical of *Staphylococcus aureus* were also noted. The average cfu count of total bacterial population and cfu count typical *of Staphylococcus aureus* was calculated from three experiments done (Table 1). The highest cfu count of 132 was observed in the plate exposed in O.P.D with 30 cfu typical *of Staphylococcus aureus,* amounting to 23% of the total bacterial population. This is followed by the plate on which thumb impression of ward boys were taken with total cfu count of 84 and cfu typical *of Staphylococcus aureus* to 12 amounting to 14% of the total bacterial population. This is followed by the plate exposed to emergency ward, corridor and patient's ward with total cfu count of 82, 23 and 11 with corresponding cfu typical *of Staphylococcus aureus* 15, 04 and 02 amounting to 18, 17 and 18% of the total bacterial population respectively. The plate exposed to O.T observed no cfu count. Thus total 63 cfu count typical of *Staphylococcus aureus* was observed from total 332 cfu amounting to 19% of the total bacterial population (Picture 1).

Phenotypic characterization of staphylococcus isolates

The colonies typical of *Staphylococcus aureus* were selected and the morphology was determined using gram stain. The characteristic gram positive cocci in clusters were observed. It was sub-cultured on milk agar for its characteristic golden yellow color appearance. The isolates which were tested for its biochemical characterization using API 20E resulted in five different biochemical patterns represented by roman figure I to V (Table 2). All isolates fermented mannitol and liquefied gelatin but no isolate was found to ferment inositol, rhamnose, melibose, sorbitol, amygladin and arabinose. Also all

Table 2: Biochemical characterization of 63 *Staphylococcus aureus* isolates recovered from different places in Jazan general hospital, Jazan, KSA.

Biochemical Reactions																			
P	T	%	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
I	32	51	-	+	+	+	-	-	+	+	+	+	-	-	-	+	-	-	-
II	17	27	-	+	+	+	+	-	+	+	+	+	-	-	-	+	-	-	-
III	08	13	-	+	+	+	-	-	+	+	-	+	-	-	-	+	-	-	-
IV	04	06	-	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-
V	02	03	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-

P = biochemical Pattern of *S. aureus* isolates; T = Total number of isolates; % = percentage of total isolates; ONPG = Beta-galactosidase activity

Abbreviations: ADH: Arginine Dihydrolase Production; LDC: Lysine Decrboxylase Production; ODC: Ornithine Decrboxylase Production; CIT: Citrate Utilization; H_2S: H_2S Production; URE: Urease production; GEL: Gelatinase production; GLU: Glucose Fermentation; MAN: Mannitol Fermentation; INO: Inositol Fermentation; SOR: Sorbitol Fermentation; RHA: Rhamnose Fermentation; SAC: Sucrose Fermentation; MEL: Melibose Fermentation; AMY: Amygdalin Fermentation; ARA: Arabinose Fermentation

Table 3: Distribution of predominant biochemical pattern *of Staphylococcus aureus* isolates from different places within Jazan general hospital, Jazan, KSA.

S.No	Place	Total cfu, typical of S.aureus	Predominant biochemical isolate of S.aureus
1	O.P.D	30	I, III and IV
2	Emergency ward	15	I and II
3	Patient's ward	02	I
4	Corridor / passage	04	I and III
5	Thumb impression of ward boys	12	I and V
6	O.T	Nil	Nil

isolates showed negative result for hydrogen sulphide production. The isolates showed varied results in fermentation of glucose and sucrose, production of urease, decarboxylation of amino acids arginine, lysine and ornithine. They also showed varied results in beta-galactosidase activity and citrate utilization. The predominant biochemical *Staphylococcus aureus* isolates from O.P.D belong to I, III and IV, emergency ward I and II, ward I, corridor I and III and Thumb impression of ward boys I and V (Table 3).

Susceptibility to selected antibiotics

The antimicrobial susceptibility tests done in triplicate of all isolates showed resistant to Bacitracin, Aztreonam, Azithromycin, and Cefixime (Table 4). However 100% susceptibility was observed against Gatifloxacin, Ampicillin sulbactam, Ciprofloxacin and Sulphamethoxazole trimethoprime. However varied susceptibility was observed for other antibiotics viz. Chloramphenicol and Cefotaxime (98%), Ofloxacin (96%), Tetracycline (95%), Vancomycin and Methicillin (93%), cefactor (85%), Cefeprime (84%), Gentamycin (82%), Latamoxef (77%) and Cefprozoil & Fusidic acid (74%). The isolates of biochemical pattern I, II, III IV and V showed varied results in its antibiotic susceptibility (Table 5) however isolate I and II were quite similar except its susceptibility towards Tetracycline and Cefaclor. Similarly isolate IV and V are similar except its susceptibility towards Tetracycline. The only isolate which found to be resistant to Methicillin was III, which however found to be sensitive to Tetracycline, Gatifloxacin, Ciprofloxacin, Vancomycin and Sulphamethoxazole trimethoprime.

Table 4: Percentage susceptibility of *Staphylococcus aureus* isolates to various antibiotics with minimum and maximum zone size and it's mean.

S. No.	Antibiotics	No. of susceptible isolates	% of susceptible isolates	Minimum zone size	Maximum zone size	Mean
1	Bacitracin 0.05 units	Nil	Nil	-	-	-
2	Gatifloxacin 5 µg	63	100	27	33	30
3	Tetracycline 30 µg	60	95	27	34	30
4	Cefotaxime Sodium 30 µg	62	98	19	29	24
5	Ampicillin Sulbactam 20 µg	63	100	20	25	22
6	Ciprofloxacin 5 µg	63	100	21	32	26
7	Aztreonam 30 µg	Nil	Nil	-	-	-
8	Ofloxacin 5 µg	61	96	19	31	25
9	Cefactor 30 µg	54	85	26	29	27
10	Azithromycin 15 µg	Nil	Nil	-	-	-
11	Sulphmethoxazole trimethoprime 25 µg	63	100	21	33	27
12	Cefprozoil 30 µg	47	74	24	31	27
13	Latmoxef 30 µg	49	77	20	28	24
14	Cefixime 5 µg	Nil	Nil	-	-	-
15	Gentamycin 10 µg	52	82	10	29	19
16	Vancomycin 30 µg	59	93	16	19	17
17	Fusidic acid 30 µg	47	74	13	19	16
18	Chloramphenicol 30 µg	62	98	27	30	28
19	Methicillin 5 µg	59	93	13	24	19
20	Cefeprime 30 µg	53	84	20	26	23

The sampling done at various places in the Jazan general hospital showed varied results in cfu count of total bacterial population as well as cfu count typical *of Staphylococcus aureus.* The count *of Staphylococcus aureus* was highest at the entry level of the patients, especially O.P.D, where more number of patients and their relatives visit the hospital. Similarly the count of *Staphylococcus aureus* was found to be more from the thumb impression of ward boys which is a matter of concern. However no count was observed in O.T which shows a great care and efficiency observed in disinfection.

The biochemical characterization of isolates by API 20E proved to be successful and five different biochemical patterns (I-V) were observed. All isolates were negative for ortho-nitrophenyl galactopyranosidase (ONPG) and positive for ADH and mannitol. Biochemical pattern I and II fermented glucose while I, II and III fermented sucrose and hydrolyze urea. The most important being all isolates were positive for mannitol which is important characteristic of pathogenic Staphylococci. These findings were similar to that of Hofherrl, lund ME [6] for *Staphylococcus aureus* causing nosocomial infection. The most predominant of all, was biochemical pattern I, which was present in all the places of the hospital where the plates were exposed. The numbers *of Staphylococcus aureus* isolates were found to be 19% of the total bacterial population in the hospital. This number is significant and can be reduced by proper disinfection.

The antimicrobial susceptibility shows varied result among different biochemical pattern, it is however important that maximum

Table 5: Distribution of biochemical and resistant pattern of *Staphylococcus aureus* isolates.

S.No	Antibiotics (µg),	I	II	III	IV	V
1	Bacitracin .05 units	R	R	R	R	R
2	Gatifloxacin 5 µg	S	S	S	S	S
3	Tetracycline 30 µg	S	R	S	R	S
4	Cefotaxime Sodium 30 µg	S	S	S	S	S
5	Ampicillin Sulbactam 20 µg	S	S	S	S	S
6	Ciprofloxacin 5 µg	S	S	S	S	S
7	Aztreonam 30 µg	R	R	R	R	R
8	Ofloxacin 5 µg	S	S	S	S	S
9	Cefactor 30 µg	S	R	R	S	S
10	Azithromycin 15 µg	R	R	R	R	R
11	Sulpha trimethoprime 25 µg	S	S	S	S	S
12	Cefprozoil 30 µg	S	S	R	S	S
13	Latmoxef 30 µg	S	S	S	S	S
14	Cefixime 5 µg	R	R	R	R	R
15	Gentamycin 10 µg	R	R	S	S	S
16	Vancomycin 30 µg	S	S	S	S	S
17	Fusidic acid 30 µg	S	S	R	S	S
18	Chloramphenicol 30 µg	S	S	R	S	S
19	Methicillin 5 µg	S	S	R	S	S
20	Cefeprime 30 µg	S	S	R	S	S

I, II, III, IV, V = Biochemical pattern of *S. aureus* isolates; R = resistant; S = susceptible.

number of isolates were susceptible to majority of antibiotics. A common pattern was observed among all isolates and they all found to be resistant to bacitracin, aztreonam, azithromycin, and cefixime. Only one isolate among biochemical pattern III was found to be resistant to methicillin, chloramphenicol, cefeprime and fusidic acid, however the same isolate is susceptible to gatifloxacin, ciprofloxacin, Vancomycin, and sulphamethoxazole trimethoprime. The antibiotics to which all isolates were susceptible are gatifloxacin, ciprofloxacin, sulphamethoxazole trimethoprime and ampicillin sulbactam which can be considered as best choice of drug as today. In the present study we found that the resistant isolate, were also susceptible to sulfamethoxazole trimethoprime and tetracycline similar to that of Raygada JL and Levine DP [7]. However chloramphenicol, ofloxacin and cefotaxime can also be considered as second line of treatment. In the present study vancomycin was also found to be effective against 93% of isolates which is considered as one of the important drug for treatment of MRSA infection, this finding correlates with that of [8]. Several newly discovered strains of MRSA show antibiotic resistance even to vancomycin. These new evolutions of the MRSA bacterium have been dubbed vancomycin intermediate-resistant *Staphylococcus aureus,* which can be treated by daptomycin and tigecycline and which do not respond to glycopeptides such as vancomycin as reported by [9]. A new antibiotic which has been identified and reported in Nature which is effective against the most resistant forms of *Staphylococcus aureus* causing nosocomial infection is platensimycin as reported by [10]. However injudicious use of these antibiotics may trigger the isolates to become resistant.

Means of prevention of spread of nosocomial infection with more resistant forms of Staphylococcus *aureus* can be possible by Patient screening upon hospital admission, with nasal cultures, prevents the cohabitation of MRSA carriers with non-carriers, and exposure to infected surfaces [11], Surface sanitizing by Alcohol or combination of alcohol and quaternary ammonium compounds extend the longevity of the sanitizing action. The prevention of nosocomial infection involves routine and terminal cleaning. Non-flammable alcohol vapor in carbon dioxide systems (NAV-CO2) do not corrode metals or plastics used in medical environments and do not contribute to antibacterial resistance. As with some other bacteria, MRSA is acquiring more resistance to some disinfectants and antiseptics. Although alcohol-based rubs remain somewhat effective, a more effective strategy is to wash hands with running water and an anti-microbial cleanser with persistent killing action, such as Chlorhexidine [12]. Used hospital gowns are associated with MRSA hospital infections, which could be avoided by proper disposal as per center for disease control CDC (1998). Glycopeptides, cephalosporins and in particular quinolones are associated with an increased risk of colonization of MRSA [13]. Reducing use of antibiotic classes which promote MRSA colonization, especially fluoroquinolones is strongly recommended.

Conclusion

The hospital sanitization should be given the first priority for control of nosocomial infections. The O.P.D, emergency wards, corridors etc should be properly cleaned and moped by disinfectant to control the spread of organism. The ward boys should wear the gloves and hand sanitizers should be installed to frequently clean the hands. The gram positive isolate especially Staphylococcus aureus which is the major cause of nosocomial infection was found to be susceptible to Gatifloxacin, Ampicillin sulbactam, Ciprofloxacin and Sulphamethoxazole trimethoprime. The only isolate which found to be resistant to Methicillin was III, which however found to be sensitive to Tetracycline, Gatifloxacin, Ciprofloxacin, Vancomycin and Sulphamethoxazole trimethoprime.

Our findings can be correlated to that of Sader H S (1) who has reported Staphylococcus aureus as one the major cause of nosocomial infection. Also only few of them were Methicillin resistant and same were susceptible to Daptomycin. In the present study the vancomycin was found to be more effective.

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