British Microbiology Research Journal
16(4): 1-9, 2016, Article no.BMRJ.27321
ISSN: 2231-0886, NLM ID: 101608140

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Molecular, Phenotypic Characterization, Antimicrobial Susceptibility Profile of Staphylococcal Strains Isolated from Preterm Neonates at Neonatal Intensive Care Unit with Clinical Characteristics and Risk Factor of these Cases

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MA, RAAED, and HEM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Author HEM managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/27321
Editor(s):
(1) Giuseppe Blaiotta, Department of Food Science, Via Università, Italy.
Reviewers:
(1) Oslinah Buru Tagutanazvo, University of Swaziland, Swaziland.
(2) Anonymous, Hungarian Academy of Sciences, Hungary.
(3) Alaa Mosbah, Mansoura University, Egypt.
(4) Masaaki Minami, Nagoya City University, Japan.
Complete Peer review History: http://www.sciencedomain.org/review-history/15864

Original Research Article

Received 28th May 2016
Accepted 13th August 2016
Published 20th August 2016

ABSTRACT

Aim of the Work: The aim of work is to study the prevalence of different staphylococcal species in preterm neonates with septicemia at neonatal intensive care unit over a period of one year and their antimicrobial susceptibility profile.
Materials and Methods: This prospective study was conducted on 80 neonates admitted to neonatal intensive care unit in Tanta University hospitals. Blood culture was done and staphylococci were isolated and identified by conventional culture methods which were confirmed by biochemical

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reactions. Antibiotic susceptibility profile of the isolated strains was done by disc diffusion method. Molecular characterization of MRSA (methicillin resistant *Staph. aureus*) strains was done by Polymerase chain reaction.

Results: Out of 80 cases of preterm infants with septicemia staphylococci were isolated from 12 cases (15%), 20 (25%) cases showed no growth of microorganisms, organisms other than staphylococci were isolated from 48 cases (60%). Of 12strains of staphylococci; 5 strains (5.25%) were identified as coagulase negative staphylococci (CoNS), 8 isolates (8.75%) were identified as *Staphylococcus aureus* which were divided as 3 strains MRSA (3.75%), and 4 stains (5%) MSSA (Methicillin sensitive *Staph. aureus*). As regard antimicrobial susceptibility profile; 60 % of MRSA strains were sensitive to meropenem, gentamicin, and ciprofloxacin, 33.3% only were sensitive to cefotaxime, vancomycin and cefepime. whereas MSSA recorded highest sensitivity to oxacillin and vancomycin (100%) while the least sensitivity was to cefepime (25%). CoNS (coagulase negative staphylococci) strains showed highest sensitivity to gentamycin (80%) and the lowest sensitivity was to vancomycin and cefepime.

Conclusion: Neonatal sepsis caused by staphylococci represents a major cause of morbidity and mortality in NICU (Neonatal Intensive care unit). Each staphylococcus species had a definite Antimicrobial susceptibility profile which must be taken in consideration before decision of the plan of antimicrobial therapy.

Keywords: Staphylococci; preterm infants; antimicrobial susceptibility.

1. INTRODUCTION

Neonatal sepsis is a clinical syndrome in an infant 28 days of life or younger, manifested by systemic signs of infection and/or isolation of a bacterial pathogen from the blood stream [1].

Neonatal Sepsis remains one of the most challenging problems. Case fatality rates now range between 5% and 60% with the highest rates reported from the lowest income countries. The World Health Organization (WHO) estimates that 1million deaths per year (10% of all under-five mortality) are due to neonatal sepsis and that 42% of these deaths occur in the first week of life [2].

The incidence of neonatal sepsis is approximately 1to10 cases per 1000 live births and 1 per 250 live premature births [3].

Staph. aureus remain a very important neonatal pathogen in developing countries, responsible for 8-22% of blood-stream isolates in different regions. *Staph. aureus* has historically plagued nurseries with numerous reported outbreaks. Since most neonatal Staphylococcal disease develop 1-3 weeks after discharge from hospital, most cases would be missed by in- hospital surveillance in developing countries. Thus, numbers reported are likely to be serious underestimates of the true burden of the disease [4].

MRSA outbreaks in Neonatal Intensive Care Units (NICUs) have been reported to be difficult to contain. Only implementation of aggressive infection control measures, with proper antibiotic therapy has been successful in controlling such outbreaks [4,5]. PCR was carried out in this study for *mecA* (for detection of methicillin resistance) and *nucA* (for detection of *Staph. aureus*).

Risk factors for CONS and methicillin resistant *Staph. aureus* (MRSA) infections include use of central venous catheters, intra-venous lipids, parenteral nutrition, mechanical ventilation, increased severity of illness and increased length of hospitalization [5].

The aim of work was to study the prevalence of different staphylococcal species in preterm neonates at neonatal intensive care unit of Tanta University Hospitals over a period of 1 year and its antimicrobial susceptibility profile and molecular characterization of MRSA in the community.

2. MATERIALS AND METHODS

This prospective study was conducted on 80 neonates admitted to neonatal intensive care unit in Tanta University hospitals in the period from April 2015 to March 2016 after approval of the ethical committee in Tanta Faculty of Medicine and a written consent from the parents of all cases.

2.1 Inclusion Criteria

Newborn infants that were admitted to neonatal intensive care unit (NICU) due to prematurity,

respiratory distress, jaundice and LBW were enrolled in this study. The neonates were selected for the study on the basis of standard clinical and laboratory criteria for diagnosis of neonatal sepsis.

2.2 Exclusion Criteria

Major congenital anomalies and Chromosomal abnormalities.

2.3 All Neonates Included in the Study were Subjected to

History taking, complete clinical examination, and routine laboratory investigation including Complete Blood Count, C- reactive protein, ESR (erythrocyte sedimentation rate), Liver& kidney functions.

Urine analysis & urine culture.

2.4 Microbiological Study

2.4.1 Blood culture

Eighty blood samples were collected from eighty neonates who were showing signs and symptoms of sepsis. Each sample was 0.5 ml of blood. Blood cultures bottles (Salix®) were used.

Subcultures on blood agar and mannitol salt agar were done, and incubated at 37℃ for another 24 hours. Characteristic staphylococcus colonies were identified by gram stain, catalase and coagulase testing according to standard bacteriological procedures.

2.4.2 Antibiotic susceptibility testing

Susceptibility testing was first performed by the standard disk diffusion method on Mueller-Hinton agar (MHA), according to the standards of Clinical and Laboratory Standards Institute (CLSI) [6]. The antibiotics chosen for the study included those commonly used in the NICU for the management of neonatal sepsis, vancomycin (10 µg), gentamicin (10 µg), oxacillin (10 µg), cefotaxime (10 µg), ciprofloxacin (10 µg), cefepime (10 µg) and meropenem (10 µg).

2.5 Polymerase Chain Reaction

Amplification of targeted genes was carried out by a polymerase chain reaction (PCR)-assay using template deoxyribonucleic acid (DNA) [7]. Bacterial DNA was extracted by using 10 mg/ml lysostaphin. PCR was carried out for *mecA* (for detection of methicillin resistance) and *nucA* (for detection of *Staph. aureus*) by using following primers *mec-A1* (5'- AAA ATC GAT GGT AAA GGT TGC C-3'), *mec-A2* (5'- AGT TCT GCA GTA CCG GAT TTG C- 3'), *nuc-A1* (5'- GCG ATT GAT GGT GAT ACG GTT-3'), *nuc-A2* (5'- AGC CAA GCC TTG ACG AAC TAA AGC- 3'. Multiplex PCR was performed for, *mecA* and *nuc A* gene in a 25 µl reaction volume (200 µlPCR vial) with 1XPCR buffer containing 10 mM Tris-HCL pH-8.3, 50 mM KCL, 1.5 mM $MgCl_2$, 200 µM concentration of each deoxynucleoside-triphosphate (dNTPs), 2.5U of taq polymerase, 0.2 µM concentration of each primer and 2.5 µl template DNA. Thermo cycling was carried out in a (Bio-metra) thermo cycler and the conditions were as follows: Denaturation at 94℃ for 10 min, followed by 30 cycles of 94℃ for 30 seconds, annealing at 59℃ for 30 seconds and extension at 72℃ for 30 seconds with a final extension of 10 min at 72℃. (Alpha Innotech Corporation U.S.A).

2.6 Statistics

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation and chi-square test by SPSS V.20.

3. RESULTS

Table 1 shows no statistical significant differences in the mean of age, gestational age, and weight of staph. Infected neonates and other neonates. Table 2 shows no statistical significant difference between staph infected neonates and other neonates as regard maternal risk factors.

Table 3 shows no statistical differences between staph. Infected neonates and other neonates as regard hypothermia, convulsions, poor suckling and poor perfusion which was higher in (Other organisms) group, but lethargy was the most common presentation on admission.

Table 4 shows no statistical significant differences as regard leucocytic count (WBC), while there were statistical significant differences between staph infected neonates and other neonates as regard hemoglobin (Hb), platelets count (PLT) and I/T ratio.

Table 5 shows no statistical significant difference between staph infected neonates and other neonates as regard CRP values.

Table 6 shows the distribution of studied neonates as regard blood culture results as the *Staph. aureus* was isolated from 12 cases (15%), other organisms were isolated from 48 cases (60%) and 20 cases (25%) showed no growth.

Regarding antibiotic sensitivity of the isolated Staphylococci in this study, most strains were sensitive to oxacillin (75%), then meropenem and gentamicin equally (66.6%), ciprofloxacin (58%), vancomycin (50%) and cefotaxime (41.6%), the least sensitivity was to cefepime (25%) (Table 7).

Out of 12 isolates of stapyylococci; the results of this study showed that the distribution of different Staphylococcal species was as following; coagulase negative staphylococci (CoNS) were isolated from 5 cases (6.25%), coagulase positive MRSA 3 cases (3.75%), and Coagulase positive non MRSA (methicillin sensitive *staph. aures* MSSA) were 4 cases (5%) (Table 8).

Regarding antibiotic sensitivity of the isolated MRSA in this study, most strains were sensitive to Meropenem, Gentamicin, and Ciprofloxacin equally (66.6%) then Cefotaxime, Vancomycin, and Cefepime equally (33.3%) while all of MRSA strains were resistant to oxacillin (100%) (Table 9).

Table 1. Demographic data of studied neonates

		Staphylococci	No growth	Other organisms	F. test	p. value
Age	Range	3 – 7	2 – 12	2 – 25	1.761	0.179
	Mean +SD	5.16 ± 1.11	5.60 ± 2.39	7.08 ± 4.71		
G. A.	Range	28 – 34	32 – 36	29 – 36	1.708	0.188
	Mean +SD	31.5 ± 2.5	34.2 ± 2.74	32.18 ± 3.07		
Wt	Range	1.1 – 2.3	1.1 – 2.4	1.1 – 2.5	1.621	0.204
	Mean +SD	1.5 ±0.694	2.33 ± 0.647	2.46 ± 0.870		

Table 2. Comparison between maternal risk factors in studied neonates

			Group			Total	X^2	P-value
			Staphylococci	No growth	Other organisms			
PROM	+ve	N	6	3	15	24	4.464	0.107
		%	50.0%	15.0%	31.3%	30.0%		
	-ve	N	6	17	33	56		
		%	50.0%	85.0%	68.7%	70.0%		
	Total	N	12	20	48	80		
		%	100.0%	100.0%	100.0%	100.0%		
Maternal UTI	+ve	N	2	0	4	6	3.123	0.210
		%	16.7%	0 %	8.3%	7.5%		
	-ve	N	10	20	44	74		
		%	83.3%	100.0%	91.7%	92.5%		
	Total	N	12	20	48	80		
		%	100.0%	100.0%	100.0%	100.0%		
Maternal Fever	+ve	N	0	1	2	3	0.577	0.749
		%	0 %	5.0%	4.2%	3.8%		
	-ve	N	12	19	46	77		
		%	100.0%	95.0%	95.8%	96.3%		
	Total	N	12	20	48	80		
		%	100.0%	100.0%	100.0%	100.0%		
No maternal R.F.	+ve	N	7	13	18	38	4.946	0.084
		%	58.3%	65.0%	37.5%	47.5%		
	-ve	N	5	7	30	42		
		%	41.7%	35.0%	62.5%	52.5%		
	Total	N	12	20	48	80		
		%	100.0%	100.0%	100.0%	100.0%		

PROM = premature rupture of membranes, UTI =urinary tract infection, R.F = risk factors

Table 3. Comparison between clinical manifestations in studied neonates

			Group			Total	X^2	P-value
			Staphylococci	No growth	Other organisms			
Hypothermia	+ve	N	1	0	6	7	2.766	0.251
		%	8.3%	0 %	12.5%	8.8%		
	-ve	N	11	20	42	73		
		%	91.7%	100.0%	87.5%	91.3%		
	Total	N	12	20	48	80		
		%	100.0%	100.0%	100.0%	100.0%		
Convulsions	+ve	N	1	1	2	4	0.331	0.847
		%	8.3%	5.0%	4.3%	5.1%		
	-ve	N	11	19	45	75		
		%	91.7%	95.0%	95.7%	94.9%		
	Total	N	12	20	47	79		
		%	100.0%	100.0%	100.0%	100.0%		
Poor Suckling	+ve	N	5	3	13	21	2.798	0.247
		%	41.7%	15.0%	27.1%	26.3%		
	-ve	N	7	17	35	59		
		%	58.3%	85.0%	72.9%	73.8%		
	Total	N	12	20	48	80		
		%	100.0%	100.0%	100.0%	100.0%		
Poor Perfusion	+ve	N	1	1	4	6	0.240	0.887
		%	8.3%	5.0%	8.3%	7.5%		
	-ve	N	11	19	44	74		
		%	91.7%	95.0%	91.7%	92.5%		
	Total	N	12	20	48	80		
		%	100.0%	100.0%	100.0%	100.0%		
Lethargy	+ve	N	7	1	18	26	11.092	0.004
		%	58.3%	5.0%	37.5%	32.5%		
	-ve	N	5	19	30	54		
		%	41.7%	95.0%	62.5%	67.5%		
	Total	N	12	20	48	80		
		%	100.0%	100.0%	100.0%	100.0%		

Regarding antibiotic sensitivity of the isolated CoNS in this study, most strains were sensitive to oxacillin (100%), Gentamicin (80%), meropenem and Ciprofloxacin (60%), Cefotaxime (40%), the least sensitivity was to Vancomycin and Cefepime (20%) (Table 10).

While Coagulase positive non MRSA (MSSA) showed high sensitivity to oxacillin and vancomycin (100%), meropenem (75%), then Gentamicin, Cefotaxime, and Ciprofloxacin (50%), the least sensitivity was to Cefepime (20%)(Table 11).

Fig. 1 shows the result of agarose gel electrophoresis of PCR amplified products with mec-A and nuc-A specifi c primers.

4. DISCUSSION

Neonatal septicemia is still a major and frequent cause of morbidity and mortality in neonatal period. Early diagnosis and treatment are critical in improving the prognosis. However clinical manifestations of neonatal septicemia are variable and non-specific, necessitating the use of laboratory tests which have an adequate degree of sensitivity and specificity so that false negative and false positive results are minimized [8].

The aim of this study is to detect the prevalence of different staphylococcal species in preterm neonates at neonatal intensive care unit of Tanta University Hospitals over a period of 1 year and its antimicrobial susceptibility profile.

In this study, Staph prevalence was low in comparison to other studies as that was done by Reda, [9] in Al- Ahrar General hospital NICU who reported that Staph incidence was 26.5%. Another study was done in Zagazig University hospital NICU by Mohamed [10] who found that Staph prevalence was 45%.

Table 4. Results of Complete Blood Count (CBC) of studied neonates

		Staphylococci	No growth	Other organisms	F. test	p. value
Hb	Range	9 – 17.9	9.30 – 19.40	6.50 – 16.50	5.293	0.007
	Mean +SD	12.93 ± 3.236	14.74 ±2.778	12.44±2.456		
Scheffe's test						
Staphylococci & no growth		**Staphylococci & other organisms**		**No growth & other organisms**		
0.183		0.852		0.007		
WBC	Range	4 – 34.20	5.80 – 13.70	5.40 – 34.20	1.529	0.223
	Mean +SD	13.12 ± 9.593	10.0±2.322	13.15 ±7.484		
0.475		1.0		0.242		
PLT	Range	55 – 360	78 – 363	22 – 431	6.346	0.003
	Mean +SD	115.91 + 82.82	234 + 85.10	163.39+103.36		
0.005		0.317		0.027		
I/ T ratio	Range	0.14 – 0.30	0.10 – 0.30	0.10 – 0.30	4.936	0.010
	Mean +SD	0.208 ± 0.046	0.161± 0.054	0.155 ± 0.053		
0.059		0.010		0.899		

Table 5. Results of C-Reactive Protein (CRP) of studied neonates

CRP	Staphylococci	No growth	Other organisms
Range	12 – 96	12 – 24	12 – 96
Mean +SD	43 ± 29.15	16.8 ± 6.57	45.2 ± 29.72
F. test	2.213		
p. value	0.119		

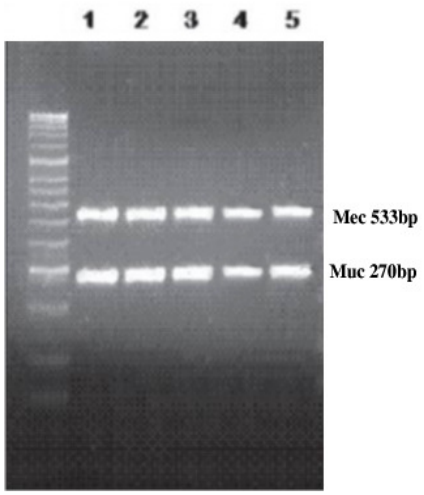

Fig. 1. Agarose gel electrophoresis of PCR amplified products with mec-A and nuc-A specifi c primers. Lane 0: 100 bp ladder and Lane 1-5: Showing mec-A gene (533 bp) and nuc-A gene (270 bp)

An incidence of 22% was observed by Macharashvilli et al. (2009) [11] in two NICUs in Georgia during a period of one year.

Salamati et al. [12] in Bahrami Children hospital in Tahran, Iran found that Staph prevalence was 30%.

Table 6. Distribution of studied neonates as regard blood culture results

Groups	Staphylococci	No growth	Other organisms
N	12	20	48
%	15 %	25 %	60 %

Table 7. Comparison between different antibiotics sensitivity and resistance in staph. infected neonates

Antibiotics	S		R	
	N	%	N	%
Meropenem	8	66.6	4	33.3
Gentamicin	8	66.6	4	33.3
Cefotaxime	5	41.6	7	58.3
Oxacillin	9	75	3	25
Vancomycin	6	50	6	50
Cefepime	3	25	9	75
Ciprofloxacin	7	58.3	5	41.6

S=sensitive, R=resistant.

In Egypt, previous studies done in Ain Shams University NICUs, by Bakry [13] NI rate was 60% and in a study done by Eliewa [14] in Ain Shams Obstetrics and Gynecology hospital NICU NI rate was 56.5%.

Also in a study by Abdel-Wahab et al. [15] in Mansora University hospital NICU, infection rate was 21.4%.

Table 8. Distribution of different *Staphylococcal* species

Groups	N	%
Coagulase –ve Staphylococci (CoNS)	5	6.25
Coagulase +ve MRSA	3	3.75
Coagulase +ve non MRSA (MSSA)	4	5

Table 9. Comparison between different antibiotics sensitivity and resistance as regarding methicillin resistant *Staph. aureus* (MRSA)

Antibiotics	S		R	
	N	%	N	%
Meropenem	2	66.7	1	33.3
Gentamicin	2	66.7	1	33.3
Cefotaxime	1	33.3	2	66.7
Oxacillin	0	0	3	100
Vancomycin	1	33.3	2	66.7
Cefepime	1	33.3	2	66.7
Ciprofloxacin	2	66.7	1	33.3

S=sensitive, R=resistant

Table 10. Comparison between different antibiotics sensitivity and resistance as regarding coagulase negative staphylococci (CoNS)

Antibiotics	S		R	
	N	%	N	%
Meropenem	3	60	2	40
Gentamicin	4	80	1	20
Cefotaxime	2	40	3	60
Oxacillin	5	100	0	0
Vancomycin	1	20	4	80
Cefepime	1	20	4	80
Ciprofloxacin	3	60	2	40

S=sensitive, R=resistant

Table 11. Comparison between different antibiotics sensitivity and resistance as regarding Coagulase positive non MRSA

Antibiotics	S		R	
	N	%	N	%
Meropenem	3	75	1	25
Gentamicin	2	50	2	50
Cefotaxime	2	50	2	50
Oxacillin	4	100	0	0
Vancomycin	4	100	0	0
Cefepime	1	25	3	75
Ciprofloxacin	2	50	2	50

S=sensitive, R=resistant

In a study in Brazil done by Couto et al. [16] infection rate was 26.5%. Jurczak et al. [17] in Poland reported a higher incidence of 38.5%. An incidence of 25.3% was observed by Babazono et al. [18] in Japan. Another study in Italy done by Orsi et al. [19] infection rate was 13.2%. In a prospective 6-year study performed by Yapicioglu et al. [20] in Turkey, the rate of infection was reported to range between 14% and 29% by years. Bolat et al. [21] reported infection rate was 16.2% in Turkey.

So, rate of infection in our hospital NICU are considered high in relation to other NICUs in Egypt and other countries.

This discrepancy between neonatal units could be possibly due to underlying differences in patient populations studied, care practices, surveillance methods and study designs [21].

In this study, Coagulase test was done for the 12 cases of staphylococci, 5 strains out of them were coagulase negative CoNS (41.6%) and 7 strains were coagulase positive which were differentiated into methicillin resistant (25%) and methicillin susceptible *Staph. aereus* (33.3%).

In this study, Among the 60 infected neonates 3 cases developed methicillin resistant *Staph. aureus* (MRSA) with an incidence of (5%) and CoNS was (8.3%) of the positive blood cultures.

This incidence was low in relation to a study done by Mahfouz et al. [22] who found that the most frequently isolated organisms were CoNS (23.4%).

Another study done by Robert et al. [23] found CoNS isolated from about 51% of blood cultures of neonates with nosocomial infection.

In another 10 year multicenter study from Australia, Isaacs [24] reported CoNS incidence of about 57%. A study performed in Turkey by Yalaz et al. [25] reported CoNS (31%) as the primary causative organism in neonatal nosocomial sepsis.

In our study the rate of MRSA (5%) is considered low when compared to other studies as the study of Babazono et al. [18] who reported that MRSA was isolated with a rate of 25.9%.

Usukura and Igarashi [26] reported that MRSA infection was observed in 38.8% among nosocomial infections in babies with very low birth weight.

Regarding antibiotic sensitivity of the isolated Staphylococci in this study, most strains were sensitive to oxacillin (75%), then meropenem and gentamicin equally (66.6%), ciprofloxacin (58%),

vancomycin (50%) and cefotaxime (41.6%), the least sensitivity was to cefepime (25%).

Regarding antibiotic sensitivity of the isolated MRSA in this study, most strains were sensitive to Meropenem, Gentamicin, and Ciprofloxacin equally (66.6%) then Cefotaxime, Vancomycin, and Cefepime equally (33.3%) while all of MRSA, strains were resistant to oxacillin (100%).

Regarding antibiotic sensitivity of the isolated CoNS in this study, most strains were sensitive to oxacillin (100%), Gentamicin (80%), meropenem and Ciprofloxacin (60%), Cefotaxime (40%), the least sensitivity was to Vancomycin and Cefepime (20%).

While Coagulase positive non MRSA (MSSA) showed high sensitivity to oxacillin and vancomycin (100%), meropenem (75%), then Gentamicin, Cefotaxime, and Ciprofloxacin (50%), the least sensitivity was to Cefepime (20%).

Bolat et al. [27] reported that *Staph. aureus* was found to be sensitive to amikacin, vancomycin, teicoplanin and linezolid.

Antibiotic use in developing countries may be influenced by factors that have little or no impact in developing countries, such as cost, parental pressure, promotion by pharmaceutical companies and lack of prescriber knowledge [28].

Despite the Egyptian guidelines indicating the need for frequent and through cleaning, bacteria were isolated from >30% of incubators in a NICU [29].

A recent review by Curtis and Shetty [30] reported that the most successful interventions to reduce hospital acquired infections were sustained hand hygiene promotion and local infection surveillance approach.

5. CONCLUSION

Neonatal sepsis caused by staphylococci represents a major cause of morbidity and mortality in NICU. Each staphylococcus species had a definite Antimicrobial susceptibility profile which must be taken in consideration before decision of the plan of antimicrobial therapy.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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