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Original article

Bacterial profile from suspected maternal sepsis and colonization cases: A step towards prevention of neonatal sepsis

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ABSTRACT

Background: Transmission of pathogens can occur through direct materno-fetal contact during antepartum period or delivery. Presence of maternal reproductive tract colonization or bacterial infections during pregnancy increases the risk of puerperal sepsis and early onset neonatal sepsis (EONS). This study was thus planned to screen the etiological agents and antibiogramfrom suspected maternal sepsis/ colonization cases.**Materials and methods:**Data was collected over a period of 1 year (September 2017- September 2018). Clinical samples-placental membrane, placental tissue, retained product of conception (RPOCs) and high vaginal swabs (HVS) received for screening of maternal sepsis or colonization were processed as per conventional microbiological techniques. Antimicrobial sensitivity was performed as per CLSI guidelines.**Result:**A total of 2405 maternal samples were included in the study. Only about 13.18% (317 samples) showed the presence of bacterial isolate, *Escherichia coli* (39%) was the predominant etiological agent isolated followed by *Staphylococcus aureus*(18%)and *Enterococcus* species (17%). There was an alarming level of drug resistance seen in both the gram positive and negative organisms.**Conclusion:**Introduction of pathogens into the female genital tract is a major risk factor for development of uterine infections and chorioamnionitis which can eventually lead to puerperal sepsis and Early onset neonatal sepsis. EONS. As seen in the present study the organisms such as *Escherichia coli* and *Staphylococcus aureus*isolated from maternal sepsis and colonization cases are the same organisms implicated from EONS.In view of increase in drug-resistant organisms prompt detection and treatment of maternal infections becomes crucial to prevent neonatal infections.

KEYWORDS:Maternal Colonization, Neonatal sepsis, Multi drug resistant

INTRODUCTION

Microorganisms that colonize the recto- vaginal and urinary system of pregnant women may be vertically transmitted to new-borns and cause neonatal sepsis. Despite the advancements in neonatal care, sepsis remains a potentially fatal condition worldwide accounting for almost 35% of neonatal deaths and 75% of the deaths within the first week of life[1].

Organisms causing early-onset neonatal sepsis are typically seen to be colonizers of the maternal genitourinary tract, leading to contamination of the amniotic fluid, placenta, cervix, or vaginal canal [2,3]. Late onset sepsis is mostly seen to be because of horizontal transmission. The organisms responsible for early onset and late onset sepsis also varies thus supporting the above said mode of transmission.

The most common microorganisms involved in Early-onset sepsis (EOS) may vary depending on time and region across the world. For instance, in western countries, Streptococcus group B is considered to be the most organism, while *Escherichia coli*, *Klebsiella*spp and *Acinetobacter*spp have been reported to be the most frequent organisms in EOS in India[4,5].

It is seen that maternal colonization during pregnancy is associated with the acquisition of organisms in the neonatal gut flora[6].Neonatal colonization with antibiotic resistant bacteria may be transient, may result in long term carriage or it may result in neonatal sepsis[7]. This may also lead to outbreaks associated with resistant organisms[8].

Sepsis due to these multidrug-resistant organisms pose a big challenge for clinicians and microbiologists as the current therapeutic options are limited [7,8]. There is an urgent need to develop newer therapeutic approaches and newer drugs to control the epidemic as a result of this MDR organism[9]. In absence of such newer treatment regimes the best practical approach available thus far is the prevention of transmission of multidrug resistant organisms.

Tracing the source and mode of transmission would help in curtailing the prevalence of infection tremendously. The present study was thus planned to study the maternal colonization and prevalence of drug resistance in these cases.

MATERIALS AND METHODS

A retrospective study was conducted in the department of microbiology in a tertiary care hospital of northern India from September 2017 to September 2018. Analysis of Clinical samples which received in laboratory such as placental tissue (retained product of conception (RPOCs) and placental membrane) and high vaginal swabs (HVS) received for maternal sepsis. A total number of 2405 samples were obtained from obstetrics and gynaecology department of our hospital.

High vaginal swab were collected with sterile swab and placental tissue was collected in sterile containers and transported immediately to the laboratory.The samples were processed in the microbiology laboratory using standard conventional microbiological techniques. Briefly the primary plating was done on blood agar and Mac Conkeys agar for swabs and of placental tissue. The placental tissue was also enriched in brain heart infusion (BHI) broth for another 24 hours and a repeat subculture was done the next day if the primary plate was sterile. The plates were incubated aerobically at 37˚C. Identification of significant isolates were done following standard microbiological techniques which involved morphological study of the colonies, Gram's staining reactions, and a battery of biochemical tests as required.

Antimicrobial susceptibility testing (AST) was performed for the pathogenic microorganisms as per CLSI guidelines .Mueller-Hinton agar (Himedia, India) was used and plates were read after 18-24 hours of aerobic incubation at 37°C. Aerobic Gram-negative bacilli were tested against cefotaxime (30μg), ceftazidime (5μg), amikacin (30μg), netilmicin (30μg), piperacillin-tazobactam(100/10μg), meropenem (10μg), imipenem (10μg) and ertapenem (10μg) using disk diffusion method. Cefotaxime-cefotaxime/clavulunate and ceftazidime-ceftazidimeclavulunate disks were used for detection of extended spectrum beta-lactamase (ESBL) production. Colistin MIC was determined using agar dilution method with concentrations of 0.25, 0.5, 1, 2, 4, 8 and 16 μg/mL.

Staphylococcus aureus isolates was tested against penicillin (10 units), gentamicin (10 μg), erythromycin (15 μg), clindamycin (2 μg), sulfamethoxazole-trimethoprim (1.25/23.75 μg), vancomycin (30 μg), linezolid (30 μg). Cefoxitin (30μg) disk was used as a surrogate for methicillin.*Staphylococcusaureus* isolates that tested resistant to cefoxitin by disk diffusion were reported as methicillin resistant *Staphylococcus aureus* (MRSA). For *Enterococcus* isolates susceptibility was done against ampicillin (10μg), high level gentamicin (120 μg), erythromycin (15 μg), vancomycin (30 μg) and linezolid (30 μg). The data was analysed using WHONET 5.6 and Microsoft excel

RESULTS

On analysis of the data obtained over a period of one-year (September 2017- September 2018) 2405 samples were collected. These included placental tissue (placental membrane and retained products of conception (RPOCs) and HVS), Table 1.

Table: 1 Distribution of samples received and samples with positive growth

Sample type	Total No. of samples obtained (N=2405)	Total No. (%) of samples positive (N=317)
Placental tissue	297	66 (20.8%)
HVS	2108	251 (79.2%)

Bacterial pathogens isolated included an array of gram positive and gram-negative bacteria. On comparison gram negative bacteria were isolated more frequently from HVS than placental tissue (67.3% vs 42.4%) whereas gram-positive bacteria were the dominant etiological group isolated from placental tissue against HVS (57.6% vs 32.7%). *Enterococcus sp.* was the most frequent organism from placental tissue (22/66, 33.33%) and *Escherichia coli* (112/251, 44.6%) was the predominant organism isolated from vaginal swabs, table 2.

A significant proportion of isolates obtained were drug resistant. Among the 317 isolates obtained 128 (40.4%) were drug resistant. All the gram negative organisms were sensitive to colistin whereas no case of vancomycin or linezolid resistance was seen amongst *Staphylococcus aureus* isolates.(table 3 and 4). High level of vancomycin resistance was seen in Enterococcus spp around 16% (table 4).

Drug resistant *Staphylococcus aureus* isolates were more common from the vaginal swabs as compared to the placental tissues whereas carbapenem resistant organisms were more common in placental tissues .(table 5).

Table: 2 Etiological agents isolated from the maternal samples

Type of bacterial pathogen isolated	Number of samples (N=317)		
	Total	HVS (N=251)	Placental tissue (N=66)
Gram positive organisms	**120 (37.9%)**	**82 (32.7%)**	**38 (57.6%)**
Staphylococcus aureus	58 (48.3%)	43(52.4%)	15(39.4%)
Beta-haemolytic *Streptococcus*	6 (5%)	5(6.1%)	1(2.6%)
Enterococcus sp	56(46.6%)	34(41.4%)	22(57.8%)
Gram negative organisms	**197 (62.1%)**	**169 (67.3%)**	**28 (42.4%)**
Escherichia coli	125 (63.4%)	112(66.2%)	13(19.6)
Klebsiellasp	37(18.7%)	32(18.9%)	5(7.5)
Citrobactersp	10(5.1)	7(4.1%)	3(4.5)
Proteus mirabilis	3(1.5%)	2(1.1%)	1(1.5)
Acinetobactersp	13(6.5%)	10(5.9%)	3(4.5)
Pseudomonas sp	9(4.5%)	6(3.5%)	3(4.5)

Table:3Antibiogram of gram negative organisms from maternal samples

	E. coli		Klebsiella sp.		Citrobacter sp.
Antibiotics	HVS N=112	Placental tissue N=13	HVS N=32	Placental tissue N=5	HVS N=7
Cefotaxime	22 (19%)	5 (38%)	8 (25%)	3 (60%)	1 (14%)
Amikacin	80 (89%)	11 (84%)	18 (56%)	3 (60%)	3 (42%)
Netilmicin	106 (94%)	13 (100%)	22 (68%)	4 (80%)	7 (100%)
Piperacillin-tazobactam	78 (69%)	11 (84%)	16 (50%)	3 (60%)	4 (57%)
Meropenem	102 (91%)	13 (100%)	18(56%)	3 (60%)	7 (100%)
Imipenem	71 (63%)	8 (61%)	15 (46%)	4 (80%)	5 (71%)
Ertapenem	78 (69%)	8 (61%)	13 (40%)	4 (80%)	5(71%)
Colistin	112 (100)	13(100)	32(100)	5 (100)	7 (100)

Table: 4Antibiogram of gram positive organisms from maternal samples

	Staphylococcus aureus		Enterococcus sp.	
Antibiotics	HVS N=42	Placental tissue N=13	HVS N=34	Placental tissue N=22
Penicillin	2 (4%)	3 (20%)	-	-
Gentamicin (10 µg)	34 (79%)	11 (73%)	-	-
Erythromycin	6 (14%)	4 (26%)	3 (8%)	1 (4%)
Clindamycin	31 (72%)	10 (66%)	-	-
Sulfamethaxazole-trimethoprim	31 (72%)	12 (92%)	-	-
Vancomycin	43(100%)	9 (60%)	26 (76%)	19 (86%)
Linezolid	43(100%)	15(100%)	33 (97%)	21 (95%)
Cefoxitin	16 (37%)	5 (33%)	-	-
Ampicillin	-	-	29 (85%)	16 (72%)
Gentamicin (120 µg)	-	-	16 (47%)	16 (72%)

Table :5Comparison of distribution of drug resistant isolates among placental tissue and HVS samples

	HVS (Number)	HVS (Percentage)	Placental tissue (Number)	Placental tissue (Percentage)
MRSA	29	67.4%	6	40%
VRE	6	17.6%	3	13.6%
ESBL	36	23.5%	5	22.7%
CRO	30	17.7%	13	46.4%

MRSA: Methicillin resistant *Staphylococcus aureus*, VRE: Vancomycin resistant *Enterococcus spp*, ESBL : Extended spectrum beta lactamase producer, CRO: Carbapenem resistant organisms

DISCUSSION

Among the 2405 samples received either for maternal genital tract colonization screening or as part of investigation for suspected maternal sepsis, 317 (13.18%) cases were culture confirmed.Introduction of pathogens into the female genital tract due to its close proximity to the peri-anal region is a major risk factor for development of uterine infections and chorio-amnionitis which can eventually lead to maternal sepsis[10,11]. Maternal sepsis is the leading cause of maternal mortality and in the developing countries the maternal mortality as a result of sepsis can be high ranging from 10-12% [11,12].

In the present study gram-negative bacteria were more commonly isolated with the prevalence being around 62% (197/317). *Escherichia coli* was the most frequent pathogen isolated (63.5%) followed by Klebsiella sp. (18.8%). Other gram negative organisms included *Citrobacter sp.* (5.1%), *Proteus mirabilis* (1.5%), *Acinetobactersps.* (6.6%) and *Pseudomonas sps.* (4.6%).

Our study is in agreement with other studies having reported a preponderance of gram negative bacteria from maternal sepsis cases with *Escherichia coli* being the most frequently isolated pathogen[10,11,13]. This could be because puerperal sepsis is commonly caused by organisms originating from genital tract infection such as E coli.

Among the 120 gram positive organisms isolated, *Staphylococcus aureus* and Enterococcus spp were almost similar in prevalence. The prevalence of *Staphylococcus aureus* was around 48.3% (58/120) followed by Enterococcus spp. at 46.7%(56/120) and least common was Group B streptococcus being around only 5%.Group B *Streptococcus* has been seen to be the most common organism in maternal genital tract colonization from developed nation[14]. But in many studies from India the most common organism isolated from maternal genital tract was *Escherichia coli* followed by gram positive organisms such as *Staphylococcus aureus*[15,16]. Better hygiene practices and maintenance of aseptic environment during parturition has led to shift in etiology of maternal sepsis in developed nations.

It is seen that transmission of organisms to neonates from mothers following materno recto vaginal colonization or maternal sepsis is the main cause of early onset neonatal sepsis[10].

Ascending infections from the mother to the foetus may occur before or during labour when colonized bacteria from the maternal perineum spread through the vaginal canal, amniotic sac, and into the once-sterile amniotic fluid[17,18].

The etiology of neonatal sepsis varies geographically and there is change in trend seen over last decade. Some studies still report gram positive organisms to be the major cause of neonatal sepsis be it early onset or late onset sepsis[19]whereas many studies have shown trend towards increase in gram negative organisms[20].

Multi-drug resistant organisms are of particular concern due to the significant morbidity and mortality associated with these infections, particularly in infants [20,21,22]. The emergence of extended spectrum beta-lactamase producing Enterobacteriaceae presents a further challenge in managing neonatal sepsis, carbapenem-resistant Enterobacteriaceae (CRE) are an emerging global public health threat[20,21,22].

There has been a steady rise in drug resistant organisms which puts an impetus on early detection and prompt treatment of the ailing mother.[21,22] In the present study a significant proportion of isolates (128 of 317, 40.4%) obtained were drug resistant. These included MRSA, VRE, ESBL and Carbapenemase producers. A significant amount of *Staphylococcus aureus*isolates were methicillin resistant (35 of 58, 60%). Vancomycin resistance was seen in 19.6% of Enterococci. Of the 197 gram negative organisms 43 (21.8%) were resistant to carbapenemswhereas 41 (20.8%) were ESBL producers. These findings reiterate the importance of multidrug resistance associated with maternal sepsis and neonatal sepsis. Antibiotic prophylaxis before labour or surgical intervention can prevent vertical transmission of pathogens to foetuses in maternal sepsis cases. This is crucial to reduce neonatal mortality and morbidity due to sepsis.

In view of the above findings it becomes imperative to screen for vaginal colonization in the antepartum period especially from patients with risk factors. This would further help in curtailing neonatal infection by instituting timely antibiotic prophylaxis to mother.

Competing interest: The authors declare that they have no competing interests.

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