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Isolation And Identification Of Gram Negative Pathogens From Sepsis Patients

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ABSTRACT

The organism most commonly Gram negative isolates were Klebsiella pneumonia organism from 10 cases of 15%, E.coli in the rate of 11.25%, 7.5% for Enterobacteriaceae and Pseudomonas putida in 2.5%. K. pneumonia has the highest sensitivity at Meropenam, E.coli was (11.25%) and the Antibiotics cephalosporins, ciprofloxacin, piperacillin and Meropenam were sensitive to all the isolates. The Antibiotic Amikacin was resistant to 32% (3/9) of isolates and 31% isolates were resistant to Ampicillin. Enterobacteriaceae was 49% sensitive to Ampicillin, 29% to Cloxacillin and Oxacillin. The P.pitida was maximum resistance to cephalosporins (65.67%) and Vancomycin (15.67%). The Antibiotics like Ampicillin, Cloxacillin and Oxacillin had resistant to the all isolates (100%). And also all isolates were sensitive to Piperacillin, Ciprofloxacin, Amikacin and Meropenam. In the Antibiotic Cephalosporins 48% resistance was observed.

Key words: Gram Negative Bacteria, Bacteremia, Antibiotic Resistant, Sepsis.

INTRODUCTION

The Sepsis is described as the infection to host response and it causes the world wide Morbidity and Mortality. The highly increased degree of Sepsis may be associated with dreadful diseases, innate catheters, antimicrobial abuse and devices of mechanics (Cunneen and Cartwright, 2004).

It is an organ dysfunction for life threatening formed by the abnormal regulation leads to infection. The absence of early detection and improper management can leads to the Septic shock, dysfunction of multiple organs and also death. It is main complicated problem of infection in the low and middle income countries and the found the neonatal Morbidity and Mortality. In the environment settings, the Sepsis frequently causes the stagnation of usual and preventable infections. Sepsis often caused infectious in health care during the delivery and affect in worldwide every year at hundreds millions of patients (McCarthy et al., 2002).

The essential stage for controlling or reducing the Sepsis infection or preventive measures includes practices for good hygiene; make certain vaccination programmes, sanitation improvement and quality and availability of water, good practices for control and prevention. Early stage detection and proper and timely management of clinical practice like ideal use of antimicrobials and fluid rescue are the possibility of living (Phillips et al., 2004). Although the inception of Sepsis can cause the little Mortality and also cause the long Morbidity needed for diagnosis and treatment. So the Sepsis is considered as a clinically collaborative approach.

The pathogens and its pattern of antimicrobial susceptibility caused the neonatal septicemia is need to choose suitable therapy of Antibiotics to reduce the Morbidity and Mortality. Geographically the Antibiotic sensitivity patterns differ based on the local pathogens and used Antibiotics in the unit of neonates. The great challenges in the management of neonatal septicemia are widespread exposure of Antibiotic resistance used. The antimicrobial susceptibility pattern and causative organisms for the neonatal septicemia are varying in their microbiological pattern (Carnevale, 2005).

The both Gram-positive and Gram-negative microbes are found in high rate of Antibiotic resistance that commonly used. The neonatal Mortality and Morbidity are related with emerged Antibiotic resistance. For both the Gram-positive and Gram-negative bacteria, the most effective drug used is Amikacin. It is used to perform the surveillance of antimicrobial susceptibility and hospital periodic review and Antibiotic national policy to decrease the load of Antibiotic resistance. The future epidemiological and clinical related researches are used to limit the modification in microbes causing neonatal Sepsis (Gupta et al., 2003). This study is an attempt to find the pathogens involved in Sepsis and their Antibiotic susceptibility in new born babies (1 to 12 months).

MATERIAL AND METHODS

Study Population: All children in the age group of 1-12 months with suspected bacteremia. By using the sterile technique about 2 ml of blood was collected (disinfected skin with alcohol and iodopovidone).

Skin Anti Sepsis
The isolated blood culture is a possible isolate than the contaminated and it depends on the level of skin anti Sepsis. The suggested antiseptics contain 70% of isopropyl alcohol, and iodine tincture or iodophor. The blood was collected by automated blood culture using the BHI broth for initial culture through syringe and inoculated under strict asepsis into bacT/ALERT culture media.

Isolation
The isolated pathogens are mainly depends on the culture. The best method is the whole blood sample is cultured in a bottle because the children rarely have anaerobic bacteremia.

Bacterial inoculation

The bacterial inoculation was streaked directly by using swab sticks in petri plates on nutrient agar and entered with date and sample code. The colonies were noted by streaked sample was incubated for 24 hours at 37˚C.

Identification of bacterial isolates

After 24 hours incubation the growth in plates were noted then the isolates were sub culture into the media plates for pure isolation. The pure culture isolation was kept in MacCartney bottles and they were applied for Gram staining, biochemical identification and morphological appearance (CLSI - Clinical and Laboratory Standards Institute, 2012).

Gram staining techniques

A small part of microbial growth were emulsified by thin smearing from the old culture colony of 18–24 hours into normal saline on a slide. The smear was heat fixed, air dried, place it to light flame and flooded with crystal violet stain for 60 seconds and kept in the staining rack carefully. For 60 seconds the Gram's iodine was added and washed gently with running water. The 70% ethanol was used as a decolouriser and stained with the safranin stain for 60 seconds, rinsed with water and dried well. Then the Gram positive organisms formed purple and the negative organisms showed the pink color under the examination of microscope.

Biochemical characterization of the isolates

The isolated organisms were identified and classified by using the following tests such as catalase test, oxidase test, Citrate utilization test, Motility test, Indole test and urease test (Jean F. Mac Faddin., 2000).

Disc Diffusion Method

Agar plate and inoculum preparation

The media used for Kirby–Bauer test is Mueller–Hinton agar (4mm), poured into petri plates of 100mm or 150mm. The pH level is 7.2 to 7.4 and the inoculum prepared is diluted a broth culture at 0.5 McFarland turbidity standard equal to 150 million cells per mL (Bauer et al., 1966).

Inoculation and incubation

By using aseptic method, the broth culture of an organism is collected with the help of sterile swab. From the swab, the excess liquid is removed by little pressing or rotating inside the tube.

In the Mueller–Hinton agar plate the swab is streaked to form a bacterial lawn. The agar plate is streaked in one direction and rotated at 120° and then rotated another 120° represents the uniform growth. The specific Antibiotics are applied in the plate contains disk using the Antibiotic disk dispenser within 15 minutes of inoculation. The forceps after flame sterilized used to press the disk into the agar and make sure it is fixed or not. Then the plates were incubated within 15 minutes of applying the Antibiotic disk at a temperature of 35 °C for overnight (NARMS, 1997).

RESULTS AND DISCUSSION

The increased degree of Sepsis and Antimicrobial resistance among the organisms in neonates born in hospital were demand to realize the earlier onset of Sepsis in organisms and to observe the prevention methods for low and middle income countries. The inadequacy of data has diminish the Sepsis of neonates is the serious problem in health of the public, the aim to observe the development of health system. In this research study, we report the rate of Sepsis occurrence, isolated organism profile and the activity of antimicrobial resistance (Ochman et al., 2000).

Culture Positivity

From the overall positive cultures, there are 70 cases were obtained among the totally 200 cases and were selected for the further study to yield the overall positivity culture rate was 25%. Paulsen et al (1993) reported the similar percentage of isolated positive blood cultures in the rate of 25% (32/128). And also the Smith et al (2002) and You et al (2006) reported the 7.9% and 12.2% percentage of culture positivity in their studies.

Organisms isolated

The organism most commonly isolated from the culture was Klebsiella pneumonia organism was the next organism isolated in the positivity of 15%. The E.coli was observed in eight children in the rate of 11.25%, five cases in the positivity rate was 7.5% for the organism Enterobacteriaceae was recorded 5% and the last rate for positivity organism was Pseudomonas putida in 2.5% of children (Table-1).

Table-1: Organisms isolated from Sepsis patients

S. No	Name of the organism isolated	No. of cases	Percentage of cases
1	Klebsiella pneumonia	10	15%
2	E.coli	8	11.25%,
3	Enterobacteriaceae	5	7.5%
4	Pseudomonas putida	2	2.5%

These findings doubtless to show the highly antimicrobial resistance in the most isolated cases among the multidrug-resistant and non multidrug - resistant pathogens. Our findings of study support the earlier studies (Waggoner et al., 2005), they reported the early onset Sepsis such as E.coli, Streptococci, Listeria monocytogenes, and Enterococcus spp. And also similar study by Viola, C., and S. J. DeVincent, 2006, found the nosocomial pathogens Acinetobacter spp, coagulase-negative staphylococci, and Klebsiella spp. on the earlier onset of Sepsis.

Gram staining and Biochemical test for the isolates

The Gram staining result for the isolated organism was Klebsiella pneumoniae is a Gram-negative, non-motile, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It was appeared as a mucoid lactose fermenter on agar. It observed the presence of catalase, citrate and urease tests (Table-2).

Table-2: Biochemical tests for the isolated organisms

S.no	Isolated organism	Gram staining	Oxidase test	Catalase test	Indole Test	Citrate utilization test	Urease test
1	Klebsiella pneumoniae	Gram Negative	-	+	-	+	+
2	E.coli	Gram Negative	-	+	+	+	-
3	Enterobacteriaceae	Gram Negative	+	-	-	-	+
4	Pseudomonas putida	Gram Negative	+	-	+	-	-

(-) negative
(+) positive

E.coli is a Gram-negative, facultative anaerobe, non sporulating coliform bacterium. Its cells were observed as rod shaped about 2.0 μm long, 0.25–1.0 μm in diameter and the volume was noted as 0.6–0.7 μm^3. It appeared in the color pink and the Catalase, citrate and indole tests were noted as positive in the biochemical tests.

The large family of Enterobacteriaceae was a Gram negative bacteria and it was observed as rod shaped in the length of 1–5 μm. In the blood agar medium, it appeared as large grey sized colonies can express pigments. It has a more flagella used for moving but few of them was non motile. The biochemical tests showed the presence of oxidase and urease test and absence of others.

The Gram negative organism Pseudomonas putida was observed as rod shaped bacteria, confirmed taxonomically as Pseudomonas species along with many groups that it lend its name. The biochemical test gives the positive results for oxidase and indole tests.

From our observation, the majority of the isolated organisms were Gram negative organisms. This observations were similar to the studies by Stokes et al (1989) isolated the common organism Salmonella

tpyhi (22.8%) followed by Staphylococcus aureus and isolated the organism Klebsiella (43%) followed by S.aureus (18.75%). Most commonly organisms isolated were differ along with mainly Gram negative organisms only. From the both of their studies they mentioned the organisms are also isolated in our observation.

Antimicrobial susceptibility tests or Antibio Gram

From the above results the most commonly isolated organism was S. epidermis were sensitive to Antibiotics Amikacin then Teicoplanin and Vancomycin. The highest sensitivity was observed against all organism was Amikacin in the range of 55/80=70% coverage. Next the maximum activity Antibiotic sensitivity was found in the Antibiotic Ciprofloxacin in the range of (53/80=67.5%) against all isolates (Table-3).

Table-3: Antimicrobial susceptibility tests for isolated pathogens

| Organism		Amikacin(AMI)	Teicoplanin(TEI)	Vancomycin(VAN)	Ciprofloxacin(CIP)	Meropenem(MER)	Piperacillin(PIP)	Fluoroquinolones(FLO)	Penicillin(PEN)	Cephalosporin(CEP)	Linezolid(LIN)	Ampicillin(AMP)	Cloxacillin(CLO)	Oxacillin (OXA)
K. pneumoniae	R	5	9	10	5	5	7	3	2	4	5	2	1	0
	S	2	2	2	2	7	6	2	1	3	3	4	2	0
E.coli	R	4	5	4	0	5	3	0	1	6	6	0	4	6
	S	3	4	4	5	9	9	5	7	2	3	0	1	0
E.bacteriaceae	R	4	14	14	8	27	42	24	12	28	20	5	0	1
	S	5	6	2	5	4	9	12	0	4	21	8	0	12
P.putida	R	0	0	2	1	5	7	0	4	3	5	0	2	0
	S	0	0	0	3	1	0	2	0	0	0	2	0	1

S-Sensitive

R-Resistance

The increased rate of early stage of Sepsis were caused due to the horizontal transmission of ultra-early from delivery rooms or vertical transmission from genital tract of maternal and they colonized with these organism after the unhygienic and obstetric practices (Biyela et al., 2004).

Klebsiella pneumoniae

The next commonly isolated organism was Klebsiella pneumoniae. The highest sensitivity was found in Meropenam and all of the isolates were sensitive to the Antibiotic Meropenam. The isolates were sensitive to Piperacillin in 81.3% and sensitive to Amikacin was 55.33% of isolates. The minimum sensitivity was found in the Antibiotic Fluoroquinolone 15.0% (2 isolates) only (fig-1). The resistance percentage was observed as 15.67% i.e. two isolates were resistant to the Antibiotic Penicillin and Cephalosporin.

**Fig-1: AntibioGram of K.pneumoniae**
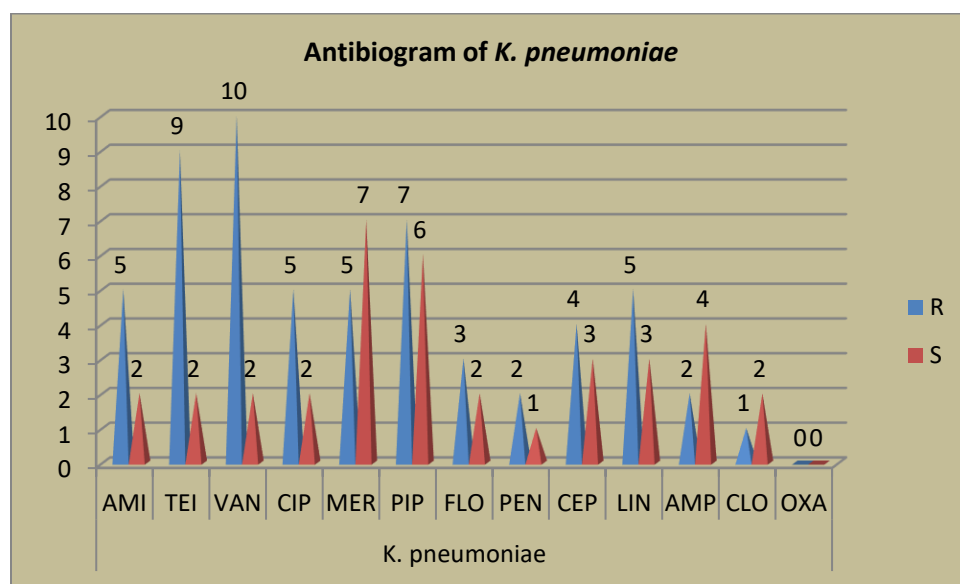
Klebsiella pneumoniae spp (15%) generate the second most common pathogen isolated in our study. Recent similar findings from India, other low and middle income countries also reported the same isolation (Cailes et al., 2015). From the 1248 isolates observed by the Network of NNPD, the leading pathogen was Klebsiella spp (32%) with the proportion of Acinetobacter spp being a low 3%. In India the tertiary neonatal increased rate of antimicrobial resistance includes Carbapenems (78·3%) and fatality rate (59%). High rate of Acinetobacter spp Sepsis leads to a dreadful threat in futures. Its indigenous is additionally facilitated control of infection practices such as hand hygiene compliance inadequately.

Escherichia coli

The culture isolates of E.coli was noted as 10 (11.25%). The Antibiotics Cephalosporins, Ciprofloxacin, Piperacillin and Meropenam were sensitive to all the isolates. ESBL have no isolate producers. The

Antibiotic Amikacin was resistant to 32% (3/9) of isolates and 31% isolates were resistant to Ampicillin (fig-2).

Fig-2: Antibio Gram of E.coli

Antibiogram of *E.coli*
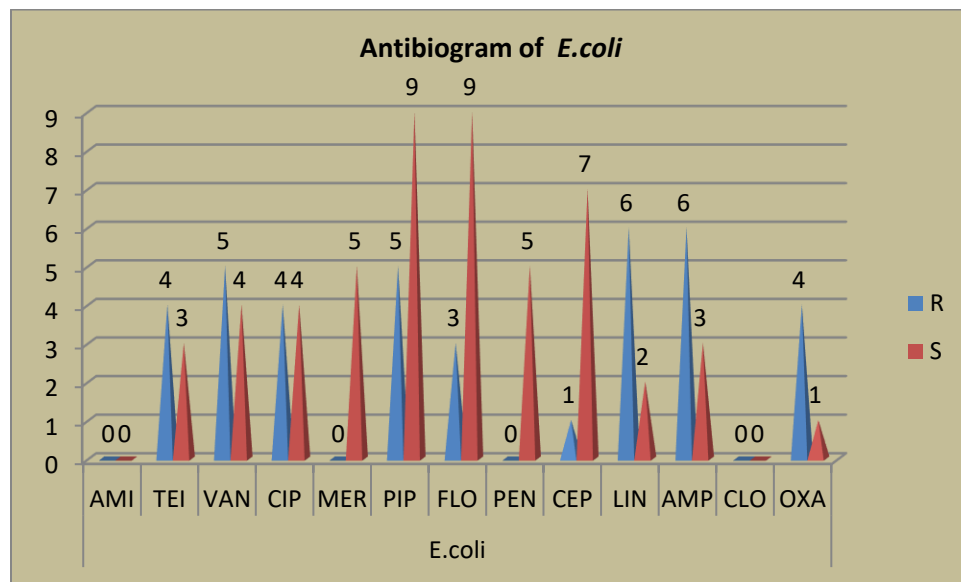
Enterobacteriaceae

The organism Enterobacteriaceae was grown in 6.8% i.e. 6 out of 80. Among them, 49% were sensitive to Ampicillin followed by 29% sensitive to Cloxacillin and Oxacillin. The maximum resistance activity was found to Cephalosporins with 65.67% and to the Vancomycin resistant-VRE (15.67%) (Fig-3). All the isolates were resistant to the Antibiotic Teicoplanin.

Fig-3: Antibiogram of Enterobacteriaceae

Antibiogram of *E.bacteriaceae*
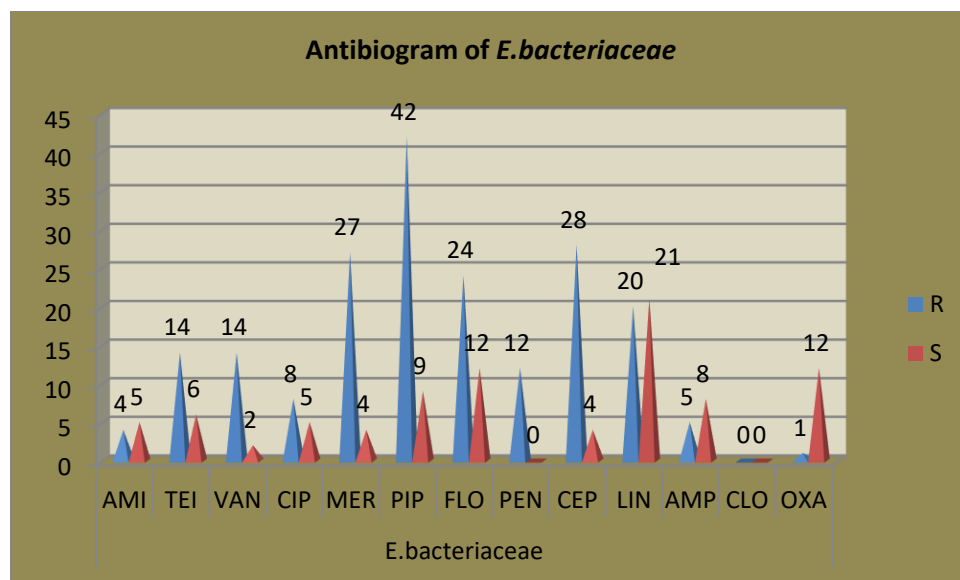
Pseudomonas putida

The immunocompromised patients (under chemotherapy) were isolated two organisms at the total growth of 1.9%. The Antibiotics like Ampicillin, Cloxacilli and Oxacillin had resistant to the all isolates (100%) (Fig-4). And also all isolates were sensitive to Piperacillin, Ciprofloxacin, Amikacin and Meropenam. In the Antibiotic Cephalosporins 48% resistance was observed.

Fig-4: Antibio Gram of P.putida

Antibiogram of *P.putida*
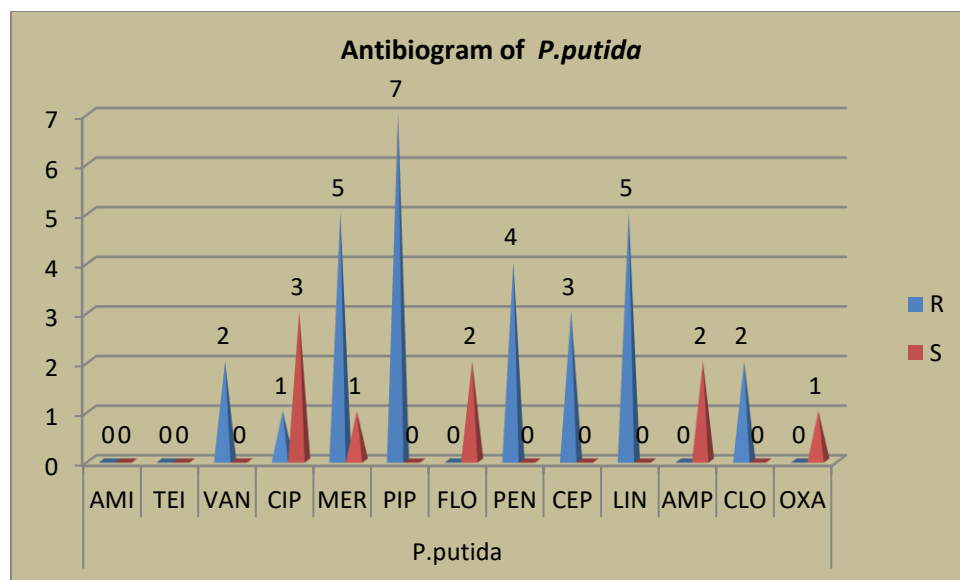
CONCLUSION

This study concludes that highly spot to undertake the research of pathogenesis on earlier onset of Sepsis and to measure the prevention of Morbidity and Mortality. And also it served as a global activity to shoot up threat Antimicrobial resistance.

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