

Antibiotic resistance pattern shown by various pathogens, causing infections in neonates

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Abstract: This study was schemed to comprehend the latest kaleidoscopic trends of bacterial resistance in neonatal pathogens against all those antibiotics commonly employed as empirical therapy in neonates. The methodological approach included; isolation and subsequent identification of those pathogens having caused bacterial infections in neonates, application of antibiotic sensitivity testing and finally construing the conclusion depicting patterns of antibiotic resistance by various pathogens, isolated from neonatal biological samples. Antibiotic resistance patterns was evident in gram-positive as well as in gram-negative bacteria in all the eight species identified in this study. Even antibiotic drugs which are being commonly relied upon for treating multi-resistant bacterial infections, found to be ineffective against many newly emerged resistant bacteria, when used alone. Resistance Antibiotics drugs against which most prominent resistance pattern emerged include; Amikacin sulphate, Linezolid, Piperacillin / Tazobactam, Amoxicillin / Clavulanic acid, Vencomycin, Cefoperazone / Sulbactam, Ceftriaxone sodium, Ciprofloxacin, Cefixime trihydrate and Imipenem. The inferred upshot suggests that antibiotic resistance is emerging fast and ever-changing phenomenon of antibiotic resistance has significantly reduced the therapeutic space to maneuver, particularly, in treating neonatal infections.

Keywords: Antibiotic resistance, antibiogram testing, empirical therapy, neonatal infections.

INTRODUCTION

Antibiotic resistance in bacterial pathogens has fully been recognized as medical catastrophe. It has reached to such a crisis proportion that global health body is consistently underscoring the need to take measures limiting its consequences. Treatment of such resistant bacterial infections with generally used antibacterials is getting complicated with each passing day (Southern and Berg, 1982). Conversely, this resistance is resulting in inapt empirical treatment, impediment in the initiating of effective therapy and the use of more toxic and less effective as well as more costly drugs (Neu, 1992). The rapid rise and spread of multi drug-resistant microbes in over the span of last two decades, has led to the development of new and effective drugs. However, such efforts remain below mark and the prospect of untreatable infections is still the haunting health issue (Sanchez *et al.*, 2000).

The antimicrobial resistance is not restricted to any specific age groups; adults, geriatric patients, women, children and neonates are all being affected by this phenomenon (Holmberg *et al.*, 1987). Add to this, the increasingly inevitable side effects of antimicrobial drugs

that are to be taken at increased doses and frequencies in order to treat such resistant infections. The apprehension is that, today, even those bacterial pathogens are manifesting resistance against once highly effective antibiotics, which cause most common and simple infections in humans; particularly staphylococci, *Klebsiella pneumonia*, *Enterococci* and *Pseudomonas* (Pfaller *et al.*, 1998).

The data concluded out of various studies conducted at a large number of hospitals, highlights that the resistance in various organisms even against effectively reliable antibiotics such as gentamicin and ampicillin is emerging fast (Raghunath, 2008). These resistant pathogens remain of a grave concern, particularly, when it comes to treating neonatal infections (Robinson and Tuovinen, 1984). The reported resistance, in neonatal infections, against third generation cephalosporins in bacteria such as *E. coli*, *Klebsiella* etc. has been well grounded by a series of studies and clinical outcomes (Blondelet-Rouault *et al.*, 1997). Similarly, resistant against Amikacin and Ceftazidime is found to be 33.9% to 80.1% respectively, while against ciprofloxacin is around 12.9% to 71.89% (Ko *et al.*, 1996).

Neonatal infections, it is estimated, cause around 1.6 million mortalities per annum in developing countries.

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Majority of these infections are being caused by those infective agents, which are increasingly developing multidrug resistance. That is why resistance even against most common antibiotics makes it a really alarming issue (Fridkin and Gaynes, 1999). What remains to be more interesting is the fact that, this resistance phenomenon is almost equally emerging in not only Gram-negative bacteria, but also in Gram-positive bacteria (Moellering Jr, 1998). It is because of such resistant organisms that the management of neonatal infections is becoming a problem in developing countries (Itokazu *et al.*, 1996). The phenomenon antimicrobial resistant is not static, rather it varies with time and space. That is why consistently periodic studies, for the evaluation of the prevalence of the extent of antimicrobial resistant, should be carried out.

In contemporary societies, this antibiotic resistance is seem to have taken dangerous trend for, it manifests itself in almost every nook and corner the globe (Kunin, 1993). An unvarying vigilance should be exercised to understand its always changing blueprints and subsequently crafting approaches to tackle this menace effectively. Keeping in view the contemporary situation of escalating antibiotic resistance and dwindling choices of treatments for these resistant infections, the present study was carried out to have a clear picture of prevalence of antibiotic resistance in bacteria that cause resistant infectious-diseases in neonates.

MATERIALS AND METHODS

For the carrying out of antibiogram testing diverse media, selective reagents, standard chemicals, antibiotic discs to check sensitivity and diagnostic kits, utilized for the carrying out of this research study, were of analytical grade (Merck Laboratory and American Sigma Laboratories). For weighing, weighing balance of analytical scale (Sartorius AG) was used. Class III biosafety cabinet of NuAir Corporate, USA was utilized. The electron microscope was of JEOL, USA. Apart from these the ancillary equipments included; autoclave, lab incubator and refrigerator (Biotechnologies Inc. USA). While chemicals used included; Normal Saline, Oxidase Reagent, Catalase Reagent, Hand Sanitizer, Gram Staining Reagents etc. The glassware utilized were of Pyrex and Jena (Germany) like petri-dishes, beakers, test tubes, glass cylinders.

Total of fifty neonates, having suspected bacterial infections were included in this study as per the inclusion criterion. Their blood and urine samples were collected after admission and immediately before the initiation of empirical treatment. The samples were sent to microbiological laboratory, where infection-causing bacterial pathogens were first isolated, identified and subsequently subjected to antibiogram testing.

Isolation of bacteria was done by using streak-plate method, while the identification was conducted by a set of methods including; gram-staining, biochemical appraisal, and concoction of pathogen-identification tests (Citrate-utilization test).

For the purpose of antibiogram testing, Kirby-Bauer Diffusion method was employed. This method ascertains the level of resistance that emerges within bacterial isolates against antibiotics used as a part of empirical treatment-regimen. In this method, pure culture of individual specie of bacterial isolate was grown on separate differential media, and then antibiotic discs were placed on the surface of culture media containing the pure population of isolated pathogen. Post-incubation analysis included measuring the ‘zone of inhibition’ around each antibiotic disc. Larger the zone of inhibition interpreted less antimicrobial resistance and vice versa. The ‘zone of inhibition’ were calculated with vernier-scale. The smaller the zone of inhibition represents the greater the level of resistant against antibiotic drug. These antibiotic-sensitivity results were further interpreted as sensitive, intermediate and resistant on the bases of the size of ‘zone of inhibition’, according to Kirby-Bauer Disk Diffusion Susceptibility Test Protocol (Bauer *et al.*, 1966).

Ethical approval statement

The research was approved by the Institutional Ethical Committee under reference No.IAEC-2016-18A.

RESULTS

Total eight species of bacterial pathogens were isolated and identified from all the biological samples. After having performed the antibiogram testing on each of these isolates, the results were compiled as mentioned below.

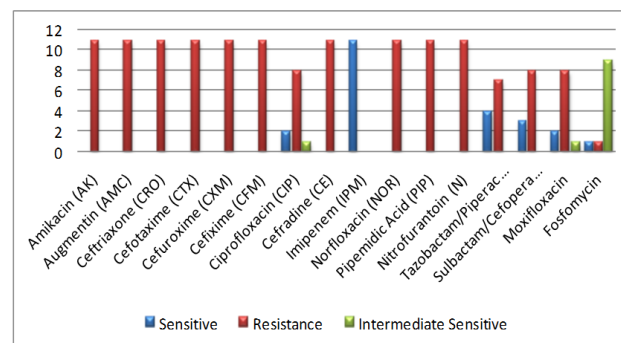


Fig. 1: Bar chart showing usceptibility pattern of *Klebsiella spp.*

Susceptibility pattern of *Klebsiella spp.*

The *Klebsiella sp.* depicted considerable resistance against most commonly used antibiotics against bacterial infections such as Amikacin, Ceftriaxone, Cefuroxime, Augmentin, Norfloxacin and Nitrofurantoin (fig. 1). The bacterial strain however came out to be sensitive against Ciprofloxacin, Moxifloxacin and Fosfomycin.

Susceptibility pattern of pseudomonas Spp.

The drugs remain effective against *Pseudomonas* include Merpenonam, Imipenem, Cefradine as shown in fig. 2. The drugs which have had intermediate efficacy in eliminating the infections caused by *Pseudomonal* strains include Moxifloxacin, Tazobactum/ Piperacillin, Ciprofloxacin and Sulbactam/ Cefoperazone. However, the high resistance has been seen emerged against Augmentin, Ceftriaxone, Cefotaxime, Cefixime and Chloramphenical.

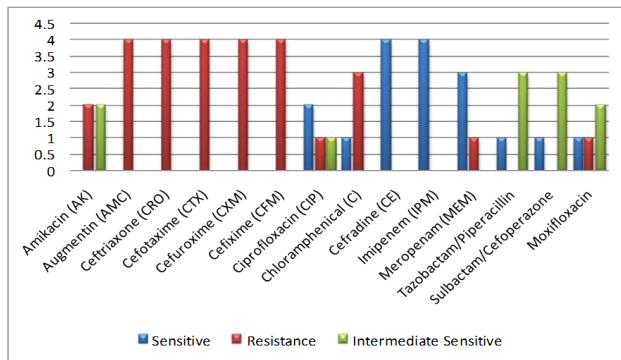


Fig. 2: Bar chart showing usceptibility pattern of *Pseudomonas* Spp.

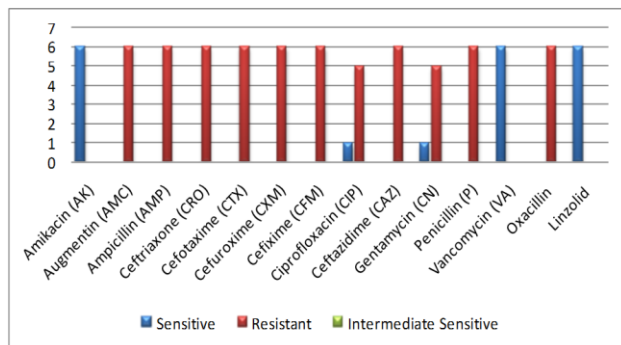


Fig. 3: Bar chart showing usceptibility pattern of *M.R.S.A*

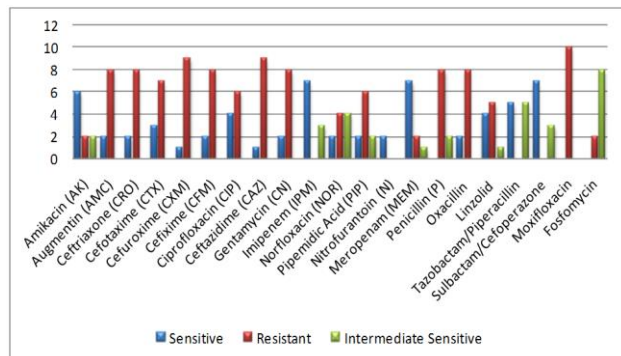


Fig. 4: Bar chart showing usceptibility pattern of *E. Coli*

Susceptibility pattern of M.R.S.A

M.R.S.A has been hold accountable for developing resistance against most of the effective antibiotics, which otherwise were used to be an inevitable part of empirical therapies. This study, too, found that only Amikacin,

Vancomycin and Linzolid are effective in treating bacterial infections in neonates. The Augmentin, Ampicillin, Ceftriaxone, Cefotaxime, Cefixime, Ceftazidime and Penicillin agents are ineffective againse bacterial infections caused by *M.R.S.A*. Only Ciprofloxacin and Gentamycin are intermediary effective as shown in fig. 3.

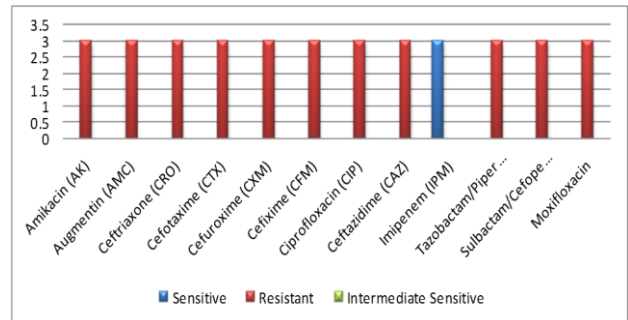


Fig. 5: Bar chart showing usceptibility pattern of *Serratia Marcescens*

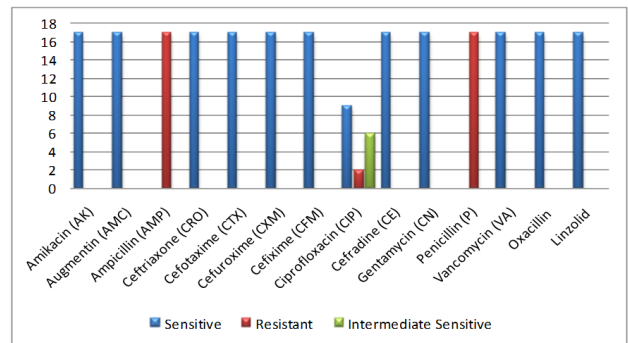


Fig. 6: Bar chart showing usceptibility pattern of *Staphylococcus* Spp.

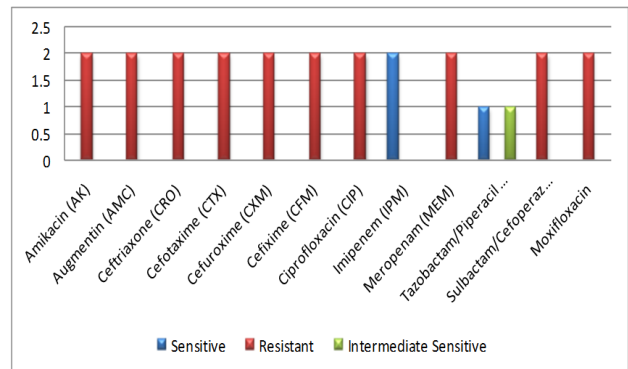


Fig. 7: Bar chart showing usceptibility pattern of *Citrobacter Freundii*

Susceptibility pattern of E. coli

E. coli is mostly resistant against Moxifloxacin and Gentamycin. The infections caused by this sole agent *E. coli* are treatable by agents such as Fosfomycin, Meropenam, Imipenem, Norfloxacin as described in fig. 4.

Table 1: Frequency of bacterial isolates

Organism	Frequency	Percent
<i>Klebsiella spp.</i>	11	22.0%
<i>Pseudomonas spp.</i>	4	8.0%
<i>M.R.S.A</i>	5	10.0%
<i>E. coli</i>	8	16%
<i>Serratia marcescens</i>	2	4.0%
<i>Staphylococcus spp.</i>	17	34.0%
<i>Citrobacter freundii</i>	2	4.0%
<i>Proteus mirabilis</i>	1	2.0%
Total	50	100%

Susceptibility pattern of *serratia marcescens*

Serratia Marcescens has acquired significant resistance against most of the antibacterial drugs which can be a part of any empirical therapy. Only Imipenem is the drug of choice in treating infections caused by this agent (fig. 5)

Susceptibility pattern of *staphylococcus spp.*

Staphylococcus species is though significantly sensitive towards Linzolid, Oxacillin, Vancomycin, Gentamycin, Cefixime, Ceftrizaxone, Cefotaxime and Amikacin, yet emerging trends show that it has completely acquiring resistance against Penicillins as is evident from fig. 6.

Susceptibility pattern of *citrobacter freundii*

The infections caused by *Citrobacter Freundii* found to be difficult to treat owing to the emerging resistance these bacteria and its strains are acquiring. Only Imepenem and Tazobactam/Piperacillin were found to be effective against infection caused by this bacteria as mentioned in fig. 7.

Susceptibility pattern of *proteus mirabilis*

The infections caused by *Proteus Mirabilis* are thought to be treated by only combination of antibacterial drugs because of the emerging resistance. *Proteus Mirabilis* is found to be resistance against Ceftriaxone, Augmentin, Cefotaxime and Cefixime (fig. 8).

DISCUSSION

Bacterial resistance is a diverse and varying phenomenon and it does not limited confine to just healthcare institutions. The spread of resistance in microbes continues both horizontally and perpendicularly in communities; aggravating the problems of treatment (Calva *et al.*, 1996). To keep abreast of the up to date and contemporary level of resistant in microorganism, there arises a need that periodic studies be carried out to understand this ever changing pattern of antibiotic resistance (Huovinen and Cars, 1998). The mortality rates exaggerate when resistant infections target neonates which already have underdeveloped immune system.

Treating resistant bacterial infections is thus of higher preference. But despite sincere efforts, the medical

fraternity is finding it difficult to treat the diverse infections with limited antibiotics (Cohen, 1992).

Apart from this, the health burden and economic fallouts of antimicrobial resistance are the other aspects which cannot be overlooked, given the limited availability of sources in developing countries. There, thus, arises an urgent need to streamline all the efforts to understanding pattern as well as mode of antimicrobial resistance in a bid to find appropriate and effective solution (Struelens, 1998). Only then, it would be wise to use the empirical treatment-regimen with sure (Kollef *et al.*, 1999).

Various antibiotics, against which varying resistance was shown by isolated pathogens include; Cefuroxime, Penicillin, Sulbactam/ Cefoperazone, Ampicillin, Tazobactam/ Piperacillin, Cefradine, Ceftriaxone, Moxifloxacin, Augmentin and Cefotexime. The pathogens against which antibiogram testing was performed include; *M.R.S.A*, *Klebsiella spp.*, *E. coli*, *Pseudomonas spp.*, *Citrobacter freundii*, *Serratia marcescens*, *Staphylococcus spp.*, and *Proteus mirabilis*.

Given the proven rising antibiotic resistance phenomenon, it is stressed that the intermittent studies should be conducted in every hospitals and health-care institutions, to understand the up to date patterns of antimicrobial resistance. This would go a long way in, not only, ensuring novel therapeutic strategies, but also help impart pragmatic treatment elements while crafting the National Health Policy for limiting resistance.

CONCLUSION

The antibiotics which came out to be still most-effective when it comes to treating the infections in neonates are; Sulbactam/ Cefoperazone, Linzolid, Amikacin and Tazobactam/ Piperacillin. However, this study found that there are antibacterial agents which considerable resistance has been developed by bacterial pathogens. Such antibiotics include Ciprofloxacin HCl, Amoxicillin/ Clavulanic Acid, Cefradine, Ceftriaxone, Imipenem, Cefotaxime Sodium, Norfloxacin, Cefuroxime Sodium, Cefixime, Fosfomycin, Pipemidic Acid and Nitrofurantoin.

From the results, it has become plainly clear that this phenomenon of antimicrobial resistance is not confined to selective bacterial pathogens, rather it persists in diverse pathogens and their species. In this study, antibiotics, the sensitivity of which were analyzed against bacterial pathogens include; Fosfomycin, Pipemidic Acid, Cefuroxime Sodium, Amoxicillin/ Clavulanic Acid, Ciprofloxacin HCl, Nitrofurantoin, Ceftriaxone Sodium, Sulbactam/ Cefoperazone, Norfloxacin, Cefotaxime Sodium, Moxifloxacin, Tazobactam/ Piperacillin, Cefixime, Imipenem and Cefradine.

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