Biocatalysis and Agricultural Biotechnology 20 (2019) 101233

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

# Emerging antimicrobial resistance causing therapeutic failure in neonatal sepsis

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ARTICLE INFO

*Keywords:*
Antibiotics
Empirical therapy
Neonatal sepsis
Microbial resistance

ABSTRACT

Neonatal sepsis maintains its rank as being one of the principal causes of neonatal morbidity as well as mortality in the developing countries. Treatment outcomes associated with neonatal sepsis continue to change with time and space. These varying trends fundamentally influenced by the resistance being emerged in neonatal pathogens which cause sepsis. This study focused on the comprehension kaleidoscopic trends of bacterial resistance in neonatal pathogens which in turn are manifesting in the form of failure of empirical antimicrobial treatment for sepsis in neonates. Our results showed that bacterial pathogens isolated found to be causing sepsis in neonates include; *Klebsiella spp, Methicillin-resistant Staphylococcus aureus (M.R.S.A), Pseudomonas aeruginosa, Staphylococcus spp, Neisseria meningitides, Streptococcus spp* and *E.Coli.* The most resistant of theses microbial isolates came out to be *Klebsiella spp* and *Pseudomonas aeruginosa.* In the current study the positive outcomes are signified by the improvements in pathological parameters as well as biochemical and physical signs and symptoms. The negative outcomes, on the other hand, are represented by deteriorating or absence of any improvement of the signs and symptoms neonates. Overall, the negative outcomes were found fifty two percent, whereas the positive outcomes were recorded as forty eight percent. This underscores the alarming trend of therapeutic failures due to emerging antimicrobial resistance in neonatal being emerged in population.

## 1. Introduction

Neonatal sepsis refers to a clinical syndrome typified by systemic signs and symptoms following bacteremia during the first few weeks of life (Odio, 1995). Despite the advances in the field of neonatal care, sepsis still adds its share greatly in an overall mortality and morbidity figures infants in Neonatal Intensive Care Units (Bizzarro et al., 2005).Clinical features of sepsis are common in neonates and a high index of suspicion is required for the appropriate diagnosis of sepsis. The blood sample is used as the benchmark for the diagnosing the sepsis universally however, culture reports takes approximately 48–72 h to be available for the initiation of definitive therapy (Sankar et al., 2008).

Sepsis in neonates is primarily caused by the gram-negative bacteria, which are becoming resistant to commonly used antibiotics (Aurangzeb and Hameed, 2003). Contemporary literature data show that neonatal infections cause mortality of about 1.7 million persons yearly in developing countries. Sepsis alone or in combination with meningitis are responsible for most of these deaths (Vergnano et al., 2005). Globally, sepsis is a major cause of death with a bulk impact on the intensive care unit (ICU) in the hospital. It has been anticipated that about 1450 patients expire in ICUs everyday due to consequence of sepsis (Kieft et al., 1993). Amongst Asian countries, Pakistan is enlisted as being the country with eighth highest rate of newborn-deaths (Shah and Padbury, 2014).

Empirical treatment is referred to the antibiotic treatment initiated before any perfect diagnosis on the basis of experience and observation. In neonates, this is frequently predictable because premature treatment with antibiotics is crucial to handle life-threatening condition (Schulz et al., 1995). However, there remain a quite small number of antimicrobial substances that can be safely employed in neonates. Also, even of these safe antibiotics, the dose adjustment is needed carefully in neonates with low birth-weight (Escobar et al., 2000). Before the

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https://doi.org/10.1016/j.bcab.2019.101233
Received 14 May 2019; Received in revised form 21 June 2019; Accepted 3 July 2019
Available online 04 July 2019
1878-8181/ 

initiation of antimicrobial treatment careful consideration is necessary to be taken into account regarding medical history, physical examination as well as laboratory data (Shaheryar et al., 2018). Antimicrobial resistance is the key phenomenon in the outcome of antimicrobial therapy in neonates. The microbial flora in the intensive care unit should also be taken into consideration because resistant infections are reportedly caused mostly by microbial flora of the care unit. That is why resistant microbial flora is being considered as the fundamental problem in bringing down the mortality and morbidity rates in neonates. The indiscriminate use of broad-spectrum antibiotics have been employed over the years to treat infections in neonates, resultantly further fanning the spread of bacterial resistance (Baltimore, 2003).

The facts in literature underscore that neonatal patients who are given ampicillin with concurrent use of cefotaxime during the first 3 days following birth is linked to greater risk of deaths, compared with the concomitant administration of gentamicin (Tzialla et al., 2015).

There are even perplexing proves whether aminoglycosides related toxicities in neonates might be circumscribed through just therapeutic drug monitoring (Brown and Campoli-Richards, 1989). The available data has no clear direction in elaborating if the effectiveness and safety of daily single dosing should be preferred over multiple daily doses of aminoglycosides and related drugs of the same class. However, what is transparently clear is the fact that these above mentioned parameters of aminoglycosides are heavily influenced by the emerging antibiotic resistance (Rybak et al., 2009).

The increasing limitations in the use of new and combinations of antibiotics to treat simple or complex infections has become a norm due to increasing antimicrobial resistance. It has become alarming situation for medical fraternity because this is leading to increase morbidity and mortality amongst neonates (Roca et al., 2015). The antibacterial resistance is therefore becoming a spotlight for the international community (Levy and Marshall, 2004).

The antimicrobial resistance phenomenon is becoming more and more ubiquitous. It no longer confines to a particular specie of bacteria; being witnessed in gram negative as well as gram positive bacterial species without any discrimination (Dijkshoorn et al., 2007). The coverage of antimicrobial resistance also varies from space to space and time to time; it is spreading in every country, community, town and street though, with varying pace (Grundmann et al., 2006). This is apparent from different studies conducted in different communities of the globe. A study conducted in the Children Hospital highlights the theme describing about 91% gram negative bacterial isolates reveal resistance toward co-amoxiclav, Amikacin and β-lactams. While, 53% of gram positive isolates depicted resistant against commonly used antibiotics such as ciprofloxacin, co-trimoxazole, penicillin and macrolides (Hannan et al., 2013).

A significant resistance has emerged in disease-causing pathogens against antibiotics such as co-trimoxazole. Similarly *Klebsiella spp.* is increasingly showing resistance against cephalosporin. The *E.coli* is not behind in spreading this resistance as it builds its ability to resist treatment of infections by cephalosporin class of drugs (Zhang et al., 2006). The widespread antimicrobial resistance entails diverse reasons. The emergence of resistance among infection-causing microorganisms, in parts, is because of the transformation in biological make up as well as transfer and spread of this mutated information to other pathogens (Bush et al., 2011). Above all, community and hospital-based spread of microbial resistance is the leading one in the list of causes of this resistance phenomenon (Gould, 1999).

This emerging trend of antimicrobial resistance is affecting the outcomes of antimicrobial therapy in neonates. If the relationship between the empirical treatment's outcomes and antimicrobial resistance is understood transparently, then it would help in crafting effective clinical strategies for treating lethal infections in neonates. This study is unique in two ways; at one hand, it takes into account the emerging trends of antimicrobial resistance in neonates; on the other hand, it brings into spotlight the relationship of the emerging resistance with the failure rates of the neonatal empirical therapy.

This study is thus intended for exploring the results of empirical therapy against sepsis in neonates as well as to find out whether antibiotic resistance has a link with treatment outcomes or not.

## 2. Materials and methods

### 2.1. Materials

Bacterial culture media; maconkey agar, blood agar, mullerhinton agar, chocolate agar, eosin methylene blue agar, simmon's citrate agar, mannitol salt agar, triple iron sugar agar, acinetobacter agar medium, christensen's urea agar, sodium azide blood agar, gram staining reagents, oxidase reagent, catalase reagent, Normal Saline, Hand Sanitizer, diagnostic kits and antimicrobial sensitivity discs used in this research study were of analytical grade (Merck Laboratory and American Sigma Laboratories). The supplementary techniques employed; autoclaving, sterilization and tantalization. Class III biosafety cabinet (NuAir Corporate, USA), Thermoelectric laboratory incubator (BINDER, Germany), the analytical weighing balance (Sartorious AG), electron microscope (JEOL, USA), and autoclaves (Memmert, Biotechnologies Inc. USA), and refrigerator (Polar King International, USA) were also used. The glassware used in the work was of Jena and Pyrex which are resistant to acid and heat. Petri-dishes, Beakers, test-tubes, and measuring cylinders were used of Pyrex (Germany). These were regularly cleaned and sterilized in hot-air oven (Memmert, USA) at 180 °C for an hour.

### 2.2. Specimen collection

Samples of blood and urine were collected before the initiation of empirical treatment via standard sampling techniques from 50 neonates for the purpose of this study. These had confirmed of carrying bacterial pathogens as per inclusion criteria of the study.

### 2.3. Isolation and identification of strains

Prior to conducting antibiogram testing, pathogenic bacteria were first isolated and subsequently identified. Isolation was done by using streak-plate and agar-plate method, while identification was conducting by a set of methods including gram-staining, biochemical appraisal, and concoction of pathogen-identification tests by utilizing a range of selective media.

### 2.4. Antibiogram testing

The Kirby-Bauer Diffusion method was employed to ascertain the level of resistance emerged within bacterial isolates against different antibiotics being used as empirical treatment regimen. In this method, pure culture of individual species of bacterial isolate was grown on specific media, and then subjected to various discs of antibiotics. 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. 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

### 2.5. Evaluation of the outcomes of empirical antibiotic therapy

The parameters on the basis of which the diagnosis of Sepsis was made for each of the neonates include intubation feeding, body temperature, abdominal distention, artificial ventilation, WBC (Neutrophil; immature-to-total) ration, Discoloration of skin, Platelet count, and whether the patient was discharged at the end of the empirical therapy or not.

The empirical treatment was assessed on the basis of progress in the pathophysiological sign and symptoms as well as to hematological parameters of patients.

Development in these parameters showed the significant suitability of the Sepsis treatment in neonates. The degree of failure or successfulness of the therapeutic management remained conditional to the worsening or effective improvements of theses parameters, respectively. The decision about what precise types of antibiotics were to administer as a part of empirical therapy was taken after the culture reports received on 48th hour or so. This decision was largely influenced by the pattern of antimicrobial resistance illustrated by the sensitivity report.

### 2.6. Statistical evaluation

The statistical software i.e. SPSS (version 21) and Graph Pad Prism was used for statistical analysis of collected data. The Chi-square test was preferred for qualitative analysis. The standard significant value remained 0.05.

## 3. Results

### 3.1. Incidence rate of sepsis alone and co-infection

Sepsis is considered to be one of the chief causes of death-tolls in neonates. The diagnosis and treatment of sepsis in neonates seek resources consumption and these result in huge economic burden on societies, not only in developed but also in developing countries. Sepsis is held responsible for heavy burden on public health in terms of treatment costs. Despite development in medico-pharmaceutical arena, the incidence of sepsis amplified even in the advanced countries over past many decades. Our results demonstrated that the neonatal patients are suffering from sepsis alone or co-existed with other infections. The incidence rate of sepsis alone stayed about 60% whereas sepsis with co-infections such as with pneumonia remained 14%, while with meningitis came out to be 4% as shown in Fig. 1.

### 3.2. Frequency of pathogens in infected neonatal patients

The results demonstrated that of the sum 50 neonates, just 43 were correctly diagnosed on the basis of the signs and symptoms at the time of admission. However, the diagnosis of the remaining seven patients was corrected at the near-end of the empirical treatment with the help of antibiogram report. Thus successful diagnosis rate found were 86%, whereas the remaining 14% considered incorrect diagnosis. Within the corrected diagnosed, about 18 (36.0%) were identified as gram-positive bacteria, while the remaining 32 (64.0%) were identified as gram-negative bacteria (Fig. 2A). Further, our results showed that eight

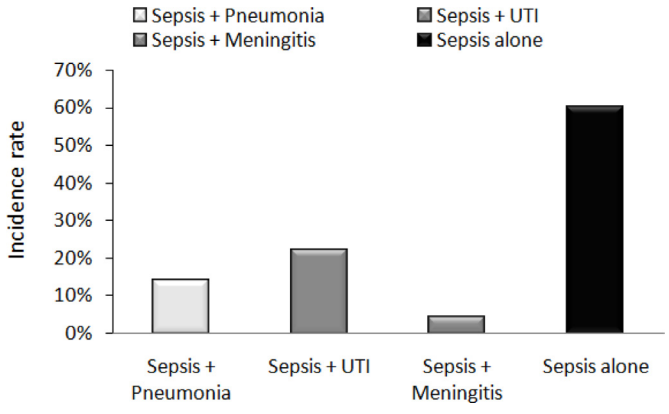


**Fig. 1.** Incidence rate of sepsis and co-infections. The bar graph showing the incidence rate of sepsis alone or in combination with other pathogens.

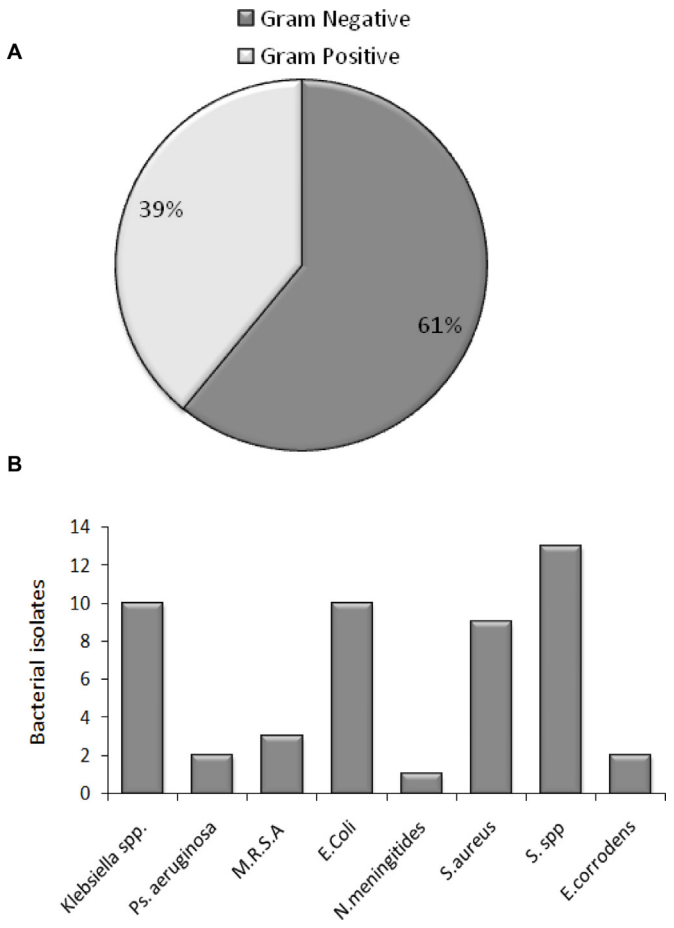


**Fig. 2.** Frequency of Sepsis and pathogens isolates. The pie chart showing frequency of diagnosis of sepsis (A). The bar chart showing frequency of bacterial isolates in different patients samples (B).

different kinds of pathogens were isolated and identified microbiologically (Fig. 2B). The highest numbers of isolates found were of *Staphylococcus spp., Klebsiella spp.,* E.coli, and *Staphylococcus aureus*.

### 3.3. Susceptibility pattern of pathogen against antimicrobial treatment

The susceptibility pattern of pathogens found to be causing sepsis with or without co-infections was evaluated. The individual pathogen's susceptibility against each of the antibiotic being used as part of empirical treatment regimen gives rise to clear picture of the role of antibacterial resistance in the failure of therapeutic outcomes. The Fig. 3A shows the susceptibility pattern (resistance, intermediate resistance and/or sensitive) of *Klebsiella spp.* Furthermore, *Pseudomonas auruginosa* was found to be sensitive only against Moxifloxacin, Tazobactum/piperacillin and Chloremphenicol. However, the antibiogram date underscores it resistance having been emerged against almost all the antibiotics included in the emirical therapeutic regimen (Fig. 3B). Various literature points to the unbiddable resistance the *M.R.S.A* strains of bacteria have adopted in adult and geriatric population. The facts remain non-contrasting in neonatal populations as highlighted by this study. The Fig. 3C underscores the clear resistance among the strains of *M.R.S.A* against antibacterial agents.

Isolates of *E.coli* showed variable pattern of sensitivity. The antibacterial agents found to have had effects against sepsis-causing microorganisms are; Linzolid, Tazobactum/piperacillin, Amikacin, Fosfomycin and Sulbactum/cefoperazone. The cephalosporins, particularly the 1st and 2nd gernerations have become unfit for treating sepsis caused by *E.coli* (Fig. 3D). Moreover, *Neisseria meningitides* are

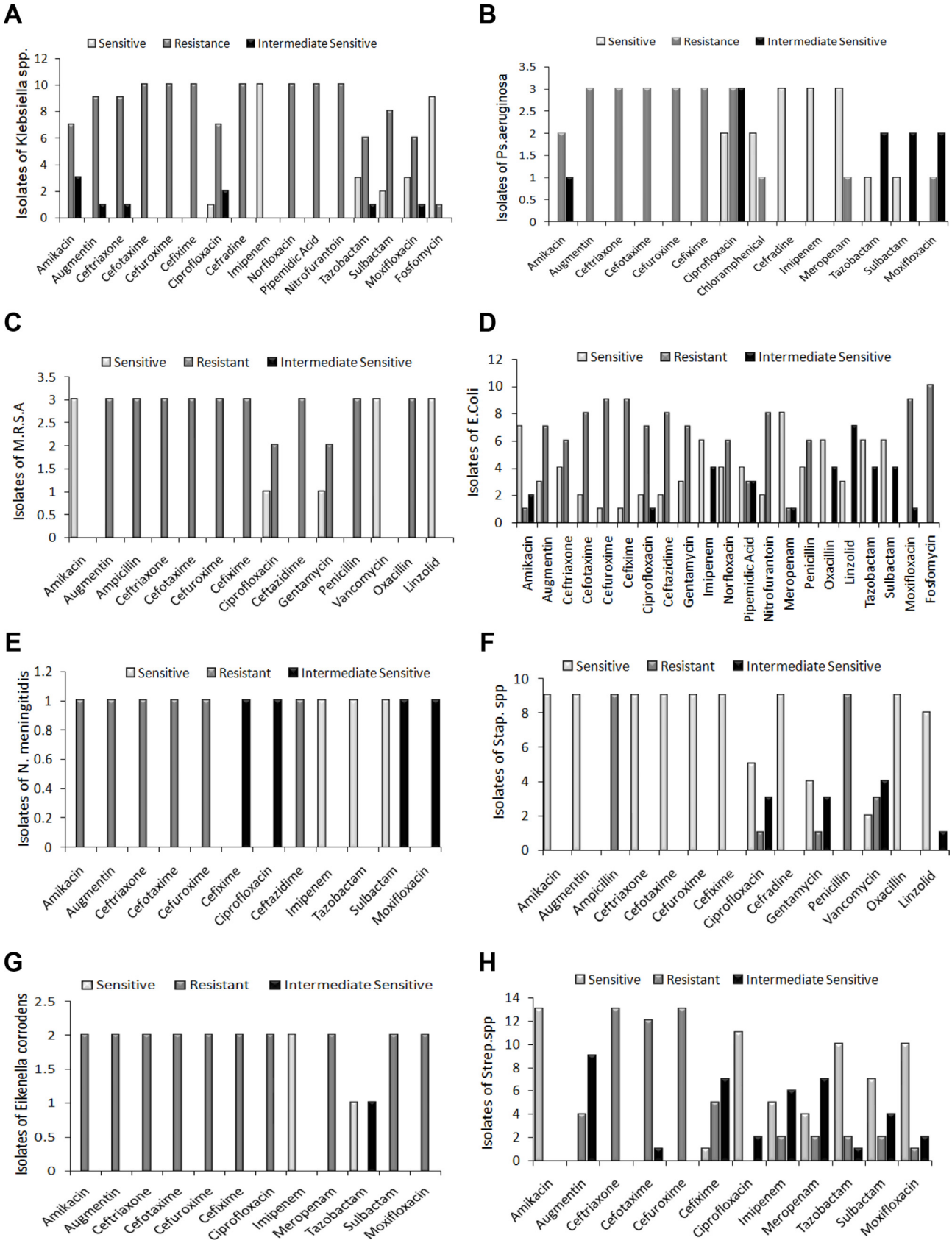


**Fig. 3.** Succeptibility of pathogens. The results showing the susceptibility pattern of *Klebsiella spp.* (A), *Pseudomonas auruginosa* (B), *M.R.S.A* (C), *E.Coli* (D), *Neisseria meningitides* (E), *Staphylococcus spp.* (F), *Eikenella corrodens* (G), *Streptococcus spp,* (H) *against antimicrobial treatments.*

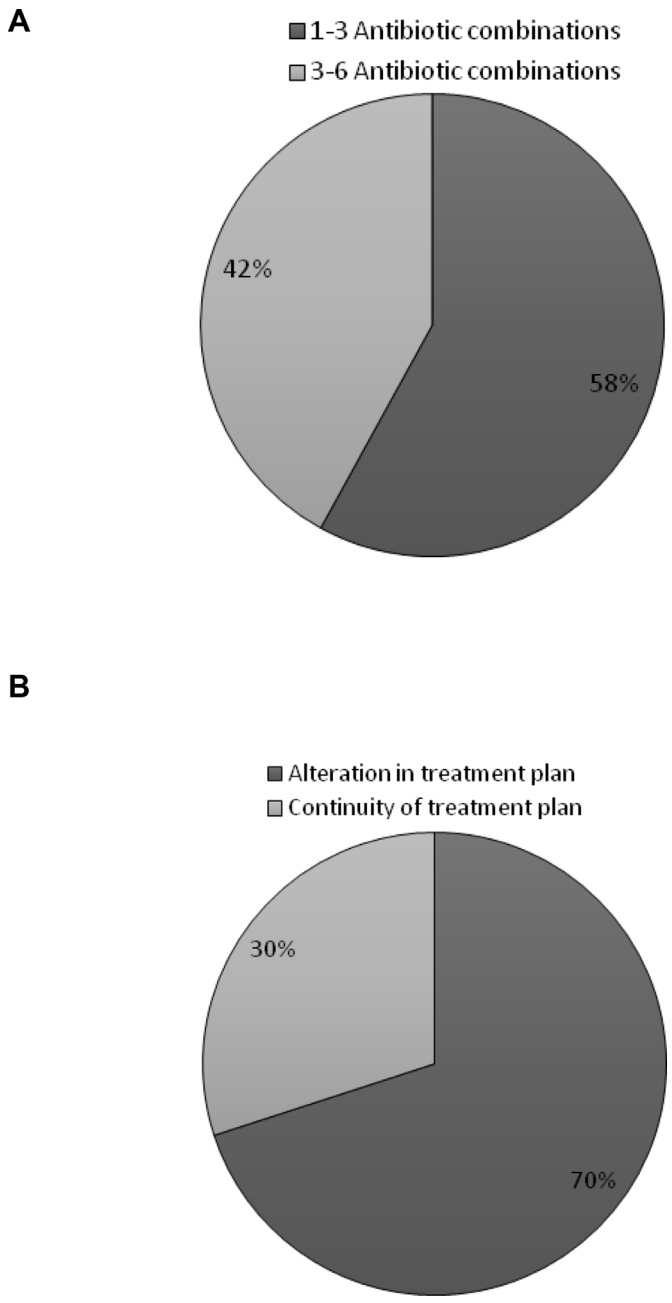


**Fig. 4.** Effect of antibiotics against bacterial infections. The results showing the frequency of use of different antibiotics in combinations (A), and the Frequency distribution of alteration of empirical treatment (B).

though uncommon strains that were found to cause sepsis in neonates, yet a significant level of resistance has been observed in this study (Fig. 3E). The strains of *Staphylococus spp.* illustrated intermediate resistance against Amikacin, Ceftrizone, Cefuroxime, Cefradine, Oxacillin, and Linzolod (Fig. 3F). This shows their increasing potential to adapt in changing environment to become more virulant and difficult-to-treat pathogens. The susceptibility pattern of the strains of Eikenellacorrodens is shown in following figure. The Fig. 3G shows clearly to what extant resistance has been acquired by these isolates. Though rare, yet deadly are these when it comes to causing highly resistant sepsis in neonates. These have key share in the overall mortality rate in neonates due to sepsis. *Streptococcus spp.* is the ones found to be more frequent in causing sepsis in neonatal population. However, a window of relief remains open as they are much less resistance against the classes of antibiotics being used as empirical treatment (Fig. 3H).

### *3.4. Antibiotics combination for the treatment of bacterial infections*

Antibiotics used as component of empirical treatment were separated into two groups. First group consisted of one-to-three antibiotics, whereas second group comprised of four-to-six antibiotics (Fig. 4A). First group has 29 patients whereas the second group entails 21. The significant number of patients were treated with four to six antibiotics, shows how difficult it has become to treat common systemic infections which were once considered to be the easy-to-treat infections. Furthermore, empirical treatment was started without any delay following the withdrawal of clinical samples from neonates to be sent to labs for microbiological assessment. The study highlighted that out of total fifty neonatal patients, only 35 cases were subjected to changes in their empirical treatment plans which were initiated after taking biological samples (Fig. 4B).

### *3.5. Comparison of pre- and post therapy to determine the improvement in patients*

The statistical data was collected before and after the initiation of empirical therapy in the form of noting signs and symptoms of the neonates. The comparison between pre- and post therapy data in turn represented the outcomes of the therapy in neonatal patients with sepsis. Given that the antimicrobial resistance is undeniably the fundamental element in affecting the outcomes of empirical treatment in neonates, thus, this study also appraised the statistics to underscore the extent of this antimicrobial resistance that could affect the outcomes of empirical treatment against sepsis. Our results showed that sepsis caused by *Klebsiella spp.* and *Pseudomonas auruginosa* remained most difficult-to-treat infections due the highest level of resistance against most of the antibiotics. The percentage progression in the essential signs and symptoms of neonates were noted in individual patients. The data was subjected to mean value (percentage) to have a clear picture of the overall improvement in biochemical and physical parameters. The improvement trend is shown in the Fig. 5A. According to this statistical data, positive outcomes came out to be 48% in sharp contrast to 52% as negative outcomes (Fig. 5B). The exaggerated percentage in the negative outcome of the empirical therapy is significantly due to the antibiotic resistance that prevails in most of the pathogens causing sepsis in neonates.

## 4. Discussion

This study included 68 neonates which were considered of having sepsis. These were scrutinized as per the inclusion criterion of this study. All these infected neonates were those admitted to “The Neonatal Ward of The Children Hospital and The Institute of Child Health”, Lahore; Pakistan for treatment. Antimicrobial testing was performed immediately following the taking of the biological samples such as blood and/or urine from the neonatal patients and sending them to microbiological laboratory. Not all the 68 neonates were able to meet the inclusion criteria. Eighteen neonates met the exclusion criteria, while the remaining fifteen fulfilled all the required standards of inclusion criteria. In essence, fifty biological samples were sent to lab for microbial isolates’ isolation and identification of their type and subsequent antibiotic sensitivity. Apart from this, during the course of pre- and post therapy time period, physical as well as pathophysiological signs and symptoms of each of the patients were recorded. The data from microbiological reports identified following eight types of isolates of having been responsible for causing sepsis in neonates; *Klebsiella Spp., Enterobacter Spp.*, *Oxacillin-resistant staphylococcus*, *Streptococcuus Spp.*, *Staphylococcus epidermidis, Escherichia Coli, Staphylococcus asreus,* and *Pseudomonas Spp*. The signs and symptoms noted before and after the empirical therapy were taken as standard for evaluating the outcome of empirical therapy. These parameters include: intubation feeding, artificial ventilation, abdominal distention, body temperature,

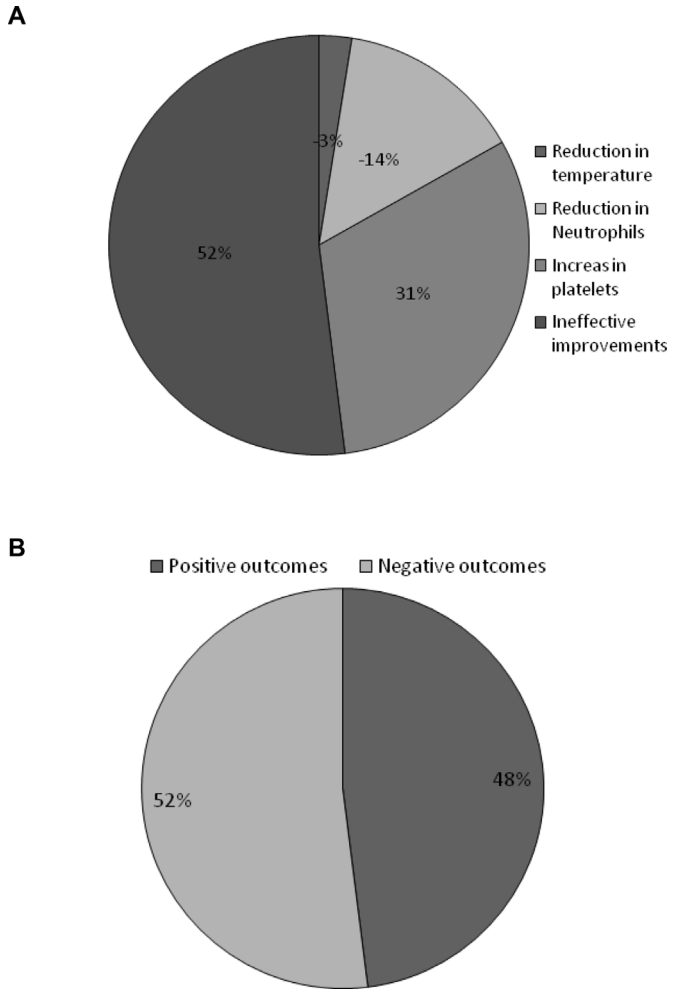


**Fig. 5.** The improvement and outcomes of treatment against Sepsis in Neonates. The pie graph results showing the percentage improvement in sign and symptoms (A). The results demonstrating the outcomes of empirical treatment against sepsis in neonates (B).

WBC ratio (Neutrophil; immature-to-total), Platelet count and presence or absence of discoloration of skin.

As per the standard rule in medical care, neonatal sepsis or sepsis-cum co-infections, the treatment includes the use of antibiotics in combination as empirical treatment. Over the years, in early-onset infections, ampicillin along with aminoglycoside has been considered as the effective empirical treatment. Same has been the rule in case of meningitis in neonates; the preferred empirical treatment remained ampicillin in combination with a drug from 3rd generation cephalosporin such as cefotaxime (Hotchkiss and Karl, 2003). In case of predictably resistant infection in neonates, vancomycin is administered along with ceftazidime ( ± an aminoglycoside). Some empirical treatments take Teicoplanin as a substitute for vancomycin (Cetinkaya et al., 2000).

These and other diverse empirical treatments give predictable results until and unless the outside factors such as antimicrobial resistance do not change. But the fact that resistance phenomenon is not static and is changing with time and place, treatment outcomes cannot be predictably same. Thus a relationship does exist between emerging antimicrobial resistance and the outcomes of institutional empirical treatments (Yalaz et al., 2006). Thus without understanding this relationship, paramount therapeutic outcomes cannot be obtained. Though developed countries have taken a great deal of steps in stressing the comprehension of this vital relationship, yet, developing countries are far behind this progressive race. That is why therapeutic outcomes in developing countries are surprisingly worse and resistance phenomenon rampant (Zaidi et al., 2005).

As the morbidity and mortality rates are the direct outcomes of the treatment's effectiveness or failure, this relationship thus becomes all the more important when it comes to treating resistant infections in neonates. The more the therapy works the lesser will be the morbidity and mortality rates, and vice versa (Weber et al., 2003). In neonatal patients, the empirical treatment is inevitable at most of the instances. The antibiotic combinations are administered on the bases of physical signs and symptoms of the diseased condition. This treatment practice is often blamed for giving undue space for the spread of resistance in bacterial isolates. Such claims in literature are justified with statistical facts of emerging infectious cases where antibiotics are no longer as effective as these were over a decade ago (Cotten et al., 2009). With time, the practice of indiscriminate use of antibiotics for the treatment of common infections is fanning the phenomenon of antimicrobial resistance (Clark et al., 2006). That is why the guidelines concerning the empirical treatment should be updated. The institutional SOPs regarding empirical treatment need to be changed, guided and added with more information (Shah and Sinn, 2012).

## 5. Conclusion

This study find out that the positive outcomes of the empirical therapy remained 48 percent owing to the increasing antimicrobial resistance being emerged and spread at institutional and community level. This is in sharp contrast with the negative outcomes of 52 percent. The decisive element in the assessment of the empirical treatment outcomes were biochemical parameters, physical signs and symptoms as well as pathological indicators. The extent of improvement in the variables were interpreted as positive outcomes, while the deterioration or aggravation of ailing signs and symptoms were made the benchmark of negative outcomes of the empirical therapy. The significant contribution in the failures of empirical antibiotic therapy against sepsis was rendered by the contemporary trends of antimicrobial resistance.

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