

## ANTIMICROBIAL ACTIVITY OF PENICILLINS IN COMMON PAEDIATRIC INFECTIONS

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### ABSTRACT

Pediatric bacterial infections are very rapidly growing in developing countries. Bacterial resistance to antimicrobial agents is a serious problem in the treatment of Pediatric bacterial infection. One of the most effective ways to control antibiotic resistance, is the development of surveillance programs. For this purpose isolates were collected from paediatric wards of different hospitals. The result shows that isolates were highly resistant against majority of selected antibiotics with increase in the MIC's. In Penicillin group, the most effective agent is Amoxicillin and Clavulanic acid, more than 90% isolates of *Staphylococcus aureus* were susceptible while other agents, as Cloxacillin, Ampicillin and Amoxicillin alone shows moderate activity against *Staphylococcus aureus* and *Escherichia coli*.

### INTRODUCTION

Pediatric is concerned with the health of infants, children and adolescents, their growth and development and of the diseases of children and their opportunity to achieve full potential as adults (Behrman *et al.*, 1998).

Pediatric bacterial infections are very rapidly growing in developing countries of the world. In Pakistan due to lack of education and socio economical condition 75% population is being in rural areas. Even in city like Karachi, there is lack of hygienic conditions and mostly contaminated water is being consumed.

Hence common pediatric bacterial infection are Diarrhea, Dysentery, Diphtheria, Cholera, Pertussis, Urinary tract infection, Gastrointestinal infection, Respiratory tract infection, Streptococcal infection, Pneumococcal infection, Meningitis, Staphylococcal infection, Tetanus and Tuberculosis (Behrman *et al.*, 1998).

The objective of the present study is to evaluate the type of organism involved in common pediatric infections in Karachi and to evaluate the resistance pattern of pediatric clinical isolate using different group of Penicillin.

### EXPERIMENTAL

#### **Agar Dilution Susceptibility Test:**

To determine the MIC for one or more bacterial isolates, the study drug may be incorporated into a liquefied agar medium (45-50°C), which is then mixed, poured into petri dishes and allowed to solidify (Barry, 1976; Snyder *et al.*, 1976). A series of petri plates are prepared with increasing concentration of the drug and with the aid of a multiple inoculum replicator (Steers *et al.*, 1959) as many as 11 different strains can be spot inoculated on to each plate. After overnight incubation, the MIC end point is read as the lowest concentration that completely inhibits growth,

disregarding a single colony or faint haze or growth (Barry, 1976; Ericson, 1971; Washington, 1985).

**Preparation of Antimicrobial Plates:**

- Dilutions of antimicrobial agents are prepared in sterile double distilled water or other appropriate diluents at a concentration 10 times that desired in the final test (Barry, 1976; Washington, 1985).
- The Agar medium is then prepared in flask or tubes and allowed to cool in a 50°C water bath.
- Sufficient volumes are prepared to fill each 9 cm petri plates with 20 to 25 ml of Agar.
- The diluted antimicrobial solutions are added to the melted and cooled medium in a ratio of 1 part antimicrobial agent to 9 part medium (2 ml of drug to 18 ml of Agar for each petri plate).
- The medium is then mixed by gently inverting the tube or flask several times. The contents are then poured into the appropriate number of petri plates.
- The plates are then set aside on a flat horizontal surface and allow to harden undisturbed.
- For reference the Agar plates should be prepared on the same day that the tests are to be performed. However for most other purposes, the antimicrobial plates can be refrigerated in a sealed plastic bag for at least 1 week without a significant loss of antimicrobial activity (Ryan *et al.*, 1970).

**Inoculation of Test Plates:**

Apply an inoculum (1-2 ml) of each organism to the surface of each antimicrobial plates with the help of a replicating device containing 11 wire loops, one for the standard and 10 for the clinical isolates. The inoculum should be applied as a spot that covers a circle about 5-8 mm in a diameter and each spot should contain about  $10^4$  viable cells (Ericson, 1971; Barry, 1976; NCCLS, 1990).

**Incubation of Test Plates:**

The inoculated plates are allowed to stand undisturbed until the spot of inoculum have absorbed completely. The plates are then inverted and allowed to incubate at 37°C for 16 to 24 hours.

Examine plate for the presence or absence of growth. The lowest concentration of each antimicrobials that inhibit growth (ignore single colony or faint inoculum haze) is considered the MIC (Wentworth, 1987).

## RESULTS AND DISCUSSION

**Table 1**  
MIC of Beta-Lactam Antibiotic *Staphylococcus aureus*

**Amoxicillin**

MIC (mg/l)	0.06	0.13	0.25	0.5	1	2
RES	98	76	63	52	43	39
SEN	2	24	37	48	57	61

**Ampicillin**

MIC (mg/l)	0.06	0.13	0.25	0.5	1	2
RES	100	93	71	54	38	32
SEN	0	7	29	46	62	68

**Cloxacillin**

MIC (mg/l)	0.06	0.13	0.25	0.5	1	2
RES	96	80	58	48	29	24
SEN	4	20	42	52	71	76

**Amoxicillin + Clavulanic Acid**

MIC (mg/l)	1	2	4	8	16	32
RES	75	48	24	14	11	6
SEN	25	52	76	86	89	94

**Ampicillin + Cloxacillin**

MIC (mg/l)	0.25	0.5	1	2	4	8
RES	83	62	54	44	31	27
SEN	17	44	46	56	69	73

**Table 2**  
MIC of Beta-Lactam Antibiotic for 84 *Escherichia coli*

**Amoxicillin**

MIC (mg/l)	2.5	5	10	20	40	80
RES	98	68	48	37	29	29
SEN	2	32	52	63	71	71

**Ampicillin**

MIC (mg/l)	2.5	5	10	20	40	80
RES	98	68	48	37	29	29
SEN	2	32	52	63	71	71

Acquisition of resistance was defined as increase in the MIC of at least 4 fold (Pour et al., 1994).  
Note Upper line of each column indicate the concentration of each drug  
Lower line indicates the number of isolates susceptible to that concentration of antibiotics.

Among semi-synthetic Penicillins, (Broad Spectrum Penicillin) Amoxicillin and Ampicillin were used in the study. Both Ampicillin and Amoxicillin were bactericidal against Gram positive and Gramnegative organism, but these drugs are destroyed by Beta-lactamase, so they were infective in most Staphylococcal infection (Dollery, 1991). Present data shows Table 1 and 2 that more than 39% and 38% isolates of *Staphylococcus aureus* and 27% and 26% of *Escherichia coli* are resistant to Amoxicillin and Ampicillin Table 1 and 2 (The MIC50 and MIC90 are  $\geq 2$ g/ml and  $>2\mu\text{g/ml}$  respectively).

The present result are in confirmation with the work of Gonzales et al. in 1995 who observed high resistance in *Staphylococcus aureus* against Ampicillin. Hugbo et al. in 1992 showed that Ampicillin was not active against *Staphylococcus aureus*. Trabuski et al. in 1988 noted the MIC of Ampicillin against *Staphylococcus aureus* was 16  $\mu\text{g/ml}$  and it inhibited 60% of the isolates.

The resistance of Amoxicillin was also reported by Catherall et al. and Wright and Reid in 1987 (The MIC of Amoxicillin was  $> 64 \mu\text{g/ml}$  against *Staphylococcus aureus*, was reported by Francioli et al. in 1991). Vu-Thien H in 1998 noted that 50 to 60% *E. coli* are resistant to Ampicillin. Lazarevic et al. in 1998 reported that in children urinary tract infections are caused by

Gram-negative bacteria in 90% case and 10% by Gram-positive bacteria. Ampicillin had a very significant role in the therapy of urinary tract infection but long term usage of Ampicillin lead to increase resistant to the drug in pediatric infection caused by *Escherichia coli*.

Vila *et al* in 1999 determined the diarrhoea caused by multidrug-resistant bacteria is an important public health problem among children in developing countries. Thirty-eight percent of the cases of diarrhoea were due to multiresistant enterotoxigenic *E. coli*, enteroaggregative *E. coli*, or enteropathogenic *E. coli*. Strains of all three *E. coli* categories showed high-level resistance to Ampicillin but were highly susceptible to quinolones. Guidelines for appropriate use of antibiotics in developing countries need updating.

Cloxacillin a Penicillinase resistant penicillin was active against both Beta-lactamase positive and negative strains or *Staphylococcus aureus* and other aerobic Gram positive cocci (Lambert and O'Grady, 1997), Akpede *et al.* 1995 indicated increase in the resistant of *Staphylococcus aureus* against Cloxacillin from 1991-1994.

The present study shows that 27% isolates of *Staphylococcus aureus* are resistant to Cloxacillin Table 1 and 2 (The MIC<sub>50</sub> and MIC<sub>90</sub> is  $\geq 2\mu\text{g/ml}$  and  $> 2\mu\text{g/ml}$  respectively). Hence the present results are in accordance with the previous studies.

#### **Combined Therapy:**

The simultaneous use of two or more antimicrobial agents have certain advantage and are recommended in specifically defined situations. Like pediatric bacterial infection, however, selection of an appropriate combination requires an understanding of the potential for interaction between the antimicrobial agents. Such interactions may have consequences for both the microorganism and the host. Since the various classes of antimicrobial agent exert different action on microorganism, one drug has the potential to either enhance or reduce the effect of the second. Presently two combination of antimicrobial were included which are very frequently used by general practitioner for the treatment. These are:

1. Amoxicillin / Clavulanic acid.
2. Ampicillin / Cloxacillin.

#### **1. Amoxicillin/Clavulanic Acid (Augmentin):**

Amoxicillin/Clavulanic acid was introduced in 1981 specifically to fight the growing problem of Beta-lactamase mediated resistance which was causing and more therapy failure with Cephalosporins and Penicillins. Clavulanic acid is available for therapeutic use only as fixed ratio combination with Amoxicillin and with Ticarcillin. In the presence of low concentration of Clavulanic acid (0.5-1mg/ml) the MIC of Amoxicillin for Beta-lactamase producing *Staphylococcus aureus* reduced 8-64 fold (Lambert and O'Grady, 1997).

Lopes *et al.* in 1992 reported 100% efficiency of Amoxicillin/Clavulanate against *Staphylococcus aureus*. Another study by Francioli *et al.* in 1990 noted MIC of Amoxicillin/Clavulanate  $8\mu\text{g/ml}$  against *Staphylococcus aureus*. Traub *et al.* in 1985 showed that the MIC was 1-16 $\mu\text{g/ml}$  against *Staphylococcus aureus*.

Lepelletier *et al.* in 1999 analyzed the epidemiology of hospital and community-acquired Pediatric infection cause by *Escherichia coli*. Of the 507 isolate of *E. coli*, 30% were resistant to Amoxicillin/Clavulanate.

Fanos and Khoory in 1999 showed that the *E. coli* was the most frequently isolated microorganism in infant and children, but not in newborn. A 3-fold increase in resistant *E. coli* isolates to Amoxicillin/Clavulanic acid was documented during the last 3 years in the pediatric population.

During present study Augementin is more active than the other antibiotics of Penicillin range. 5% isolates of *Staphylococcus aureus* shows resistance Table 1 and 2 (The MIC50 is  $\leq 4\mu\text{g/ml}$  and MIC90 is  $> 32\mu\text{g/ml}$ ).

## 2. Ampicillin/Cloxacillin (Ampiclox):

Inadequate binding of Cloxacillin to Staphylococcal Beta-lactamase was a major reason for failure to demonstrate Ampicillin-Cloxacillin synergism against *Staphylococcus aureus* (Sabath et al., 1963).

Present result shows that there is no improvement in antimicrobial activity of Amicillin when it combine with Cloxacillin. Nearly 27 isolates of *Staphylococcus aureus* shows resistance against Ampicillin and Cloxacillin combination. (Table 1 and 2 (MIC50 is  $8\mu\text{g/ml}$  and MIC90 is  $> 8\mu\text{g/ml}$  respectively).

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