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# ANTIBIOTIC RESISTANCE AND PLASMID ANALYSIS OF BACTERIA ISOLATED FROM CHILDREN WITH BACTEREMIA AND MALARIA IN EKITI STATE

Microbiology

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## ABSTRACT

Increased resistance of bacteria isolated from children with bacteremia and malaria has being reported to be due to lack of laboratory investigations before antibiotics are prescribed alongside antimalaria. Therefore this study was carried out to determine the rate of resistance of bacteria isolated from children with bacteremia and malaria. A total of 34 bacteria strains belonging to 4 genera were isolated out of which 44.1% were E. coli, 29.4% were Staphylococcus aureus, 17.7% were Pseudomonas aeruginosa and 8.8% were Salmonella typhi. Fourteen (7.5%) of the children had concomitant bacteremia and malaria, Thirty seven (19.9%) had malaria only while 20 (10.8%) had bacteremia only. Twelve (80%) of the isolated E.coli exhibited the highest resistance to augmentin while 6(60%) of the staph aureus exhibited the highest resistance to augmentin and ceftriaxone. Two (66.6%) of the salmonella typhi showed the highest resistance to ampicillin and ceftriaxone, while 3(50%) of the pseudomonas exhibited the highest resistance to gentamicine. The plasmid analysis of the selected antibiotic resistance bacteria shows that there are detectable plasmid in 3(37.5%) of the isolates. The post curing of the isolates containing plasmid shows that the resistance in E.coli was not plasmid mediated while the resistance in Staphylococcus aureus was plasmid mediated. In conclusion the result shows that the rate of antibiotic resistance is high among the children examined and it is not all the children who have malaria that also have bacteremia, therefore a proper laboratory test should be carried out before antibiotics is accompanied by antimalaria.

## KEYWORDS

Antibiotic resistance, Plasmid, Children, Bacteremia, Malaria

## INTRODUCTION

Antibiotic resistance is one of the most serious public health concerns worldwide and is increasing at an alarming rate, making daily treatment decisions more challenging. The rate of resistance of bacteria associated with bacteraemia to commonly prescribed antibiotics also poses a major challenge to the treatment and management of this infection. It is a common practice especially among children to accompany antimalaria drugs with antibiotics even when there is no laboratory evidence of bacterial infection. This leads to unguided empirical treatment with antibiotics, which, in turn, may result into an increase of antimicrobial resistance (Hausdorff *et al*., 2001). Increased resistance to Gentamicin, Amoxicillin/Ampicillin, Ceftazidime and Cefuroxime has been found to be due to non-investigation of children put on antibiotics, unjustified and prolonged use of antibiotics, lack of utilisation of laboratory investigations and delay in obtaining laboratory results (Musoke and Revathi, 2000).

Most a times, when a bacterial infection is assumed, due to inadequate laboratory capacity to conduct culture and sensitivity, treatment is often given in the form of broad-spectrum antibiotics which increase the rate of resistance (Anstey *et al*., 2012).

In areas where malaria is endemic, WHO advises that a presumptive diagnosis should be made (WHO, 2005) which is often accompanied by antibiotics. This may also contribute to the development of antibiotic resistance in bacteria associated with bacteremia.

## MATERIALS AND METHODS

Part of this work has being published by Oluyege *et al.,* (2017). Antibiogram was performed for all isolates using the disk diffusion method and the results were interpreted using the criteria of the Clinical Laboratory Standard Interpretation (CLSI) (2012). Acetone-alkaline hydrolysis method of plasmid analysis was carried out on some of the bacterial isolates that showed antibiotic resistance according to Kraft *et al.*, (1988). Curing of the plasmid was done to determine whether or not a plasmid encode a trait that codes for multiple antibiotic resistance. Curing was done using sodium deodecycl sulphate (SDS) according to Kraft *et al.,* (1988)

## RESULTS

A total of 34 bacteria strains belonging to 4 genera were isolated out of which 44.1% were *E. coli,* 29.4% were *Staphylococcus aureus*, 17.7% were *Pseudomonas aeruginosa* and 8.8% were *Salmonella typhi* (Table 1). Fourteen (7.5%) of the children had concomitant bacteraemia and malaria, 37 (19.9%) had malaria only, while 20 (10.8%) had bacteraemia only (Table 2).

Eleven (73.3%) of the isolated *E. coli* were resistant to cefuroxime while 12 (80%) were resistant to augmentin, the least resistant was towards gentamicine with 5 (33.3%). Six (60%) of the *Staphylococcus aureus* isolated were resistant to augmentin and ceftriaxone , while 5 (50%) were resistant to cefixime and gentamicine. 2 (66.6%) of *Salmonella typhi* were resistant to ampicillin and ceftriaxone with 0% resistance to cefuroxime and augmentin. 3 (50%) of *Pseudomonas* were resistant to gentamicine with 2 (33.3%) resistant to cefuroxime ,ampicillin and cefixime. (table 3).

**Table 1: Profile of Bacteria Isolated from Children Examined for Bacteraemia Concomitant with Malaria**

| Organisms | Occurence | % |
|---|---|---|
| E.coli | 15 | 44.1 |
| Staphylococcus aureus | 10 | 29.4 |
| Pseudomonas aeruginosa | 6 | 17.7 |
| Salmonella typhi | 3 | 8.8 |
| Total | 34 | 100 |

**Table 2: Prevalence of Bacteraemia and Malaria Infections in Children Examined for Bacteraemia Concomitant with Malaria**

| Type of Infections | Occurence | % |
|---|---|---|
| Bacteraemia only | 20 | 10.8 |
| Bacteraemia concomitant with malaria | 14 | 7.5 |
| Malaria only | 37 | 19.9 |
| Negative | 115 | 61.8 |
| Total | 186 | 100 |

**Table 3: Antibiotic resistance of *E.coli isolated from children with bacteremia and malaria***

| Antibiotics | E.coli(n=15) | staph aureus (n=10) | salmonella typhi(n=3) | pseudomonas(n=6) |
|---|---|---|---|---|
| Cefuroxime | 11(73.3%) | 4(40%) | 0(0%) | 2(33.3%) |
| Ampicillin | 9 (60.0%) | 4(40%) | 2(66.6%) | 2(33.3%) |
| Gentamicine | 5 (33.3%) | 5(50%) | 1(33.3%) | 3(50.0%) |
| Augmentine | 12(80.0%) | 6(60%) | 0(0%) | 2(33.3%) |
| Cefixime | 6 (40.0%) | 5(50%) | 1(33.3%) | 2(33.3%) |
| Ceftriaxone | 10(66.6%) | 6(60%) | 2(66.6%) | 1(16.7%) |

The plasmid analysis of the selected antibiotic resistance bacteria shows that there are detectable plasmid in 3(37.5%) of the isolates which are represented by bands (figure 1)

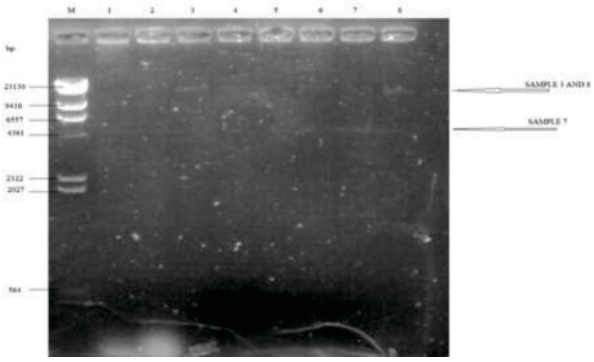


**Figure 1: Detection of Plasmid in bacteria that showed Multiple Antibiotics Resistance in Children Examined for Bacteraemia with Malaria**

Lane 1,2 ,3 and 4 represent *E. coli*, lane 5 and 6 represent; *Pseudomonas aeruginosa* ,Lane 7 and 8 represent *Staphylococcus aureus*.

The post curing of the isolates containing plasmid shows that the resistance in *E.coli* was not plasmid mediated as the bacteria was still resistant to the same antibiotics after the plasmid has been removed. Meanwhile the resistance in *Staphylococcus aureus* was plasmid mediated as the bacteria were sensitive to the antibiotics previously used after plasmid has been removed as shown in table 4

**Table 4: Pre and Post Curing Antibiogram of the bacteria Isolated from Children Examined for Bacteraemia with Malaria**

| | Antibiotics | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CEF | | AMP | | GEN | | AUG | | CRO | | CFM | |
| Isolates | Before Curing Mm | After Curing Mm | Before Curing (mm) | After Curing (mm) | Before Curing (mm) | After Curing (mm) | Before Curing (mm) | After Curing Mm | Before Curing Mm | After Curing Mm | Before Curing (mm) | After Curing |
| Sample 3(E. coli) | 10(R) | 12(R) | 5(R) | 5(R) | 5® | 5(R) | 10 (R) | 12 (R) | - | - | 10 (R) | 10(R) |
| Sample 7(Staph) | - | - | 0(R) | 10(R) | 12 (R) | 18 (S) | 10 (R) | 21(S) | 10 (R) | 20 (S ) | 14 (R) | 20 (S) |
| Sample 8(Staph) | 5® | 18 (S ) | 0® | 21 (S ) | 12 (R) | 21 (S) | - | - | 10(R) | 18 (S) | 14 (R) | 18(S) |

*E .coli*: not plasmid mediated
*Staphylococcus aureus:* Plasmid mediated
Key: R; resistance, S; sensitive
CEF: cefuroxime, AMP: ampicillin, GEN: gentamicin, CRO: ceftriaxone, AUG: augmentin, CFM: cefixime

## DISCUSSION

Antibiotic resistance is one of the most serious public health concerns worldwide and is increasing at an alarming rate, making daily treatment decisions more challenging. Most times antibiotics are prescribed alongside antimalaria and often without a laboratory test. However as shown in this study, not all the children who have malaria also have bacteremia but bacterial culture to diagnose bacterial infection is not routinely done in most of the primary and secondary health facilities. This leads to unguided empirical treatment with antibiotics, which, in turn, may result into an increase of antimicrobial resistance (Hausdorff *et al*., 2001) ,destruction of the normal flora and even make the children more susceptible to other infections. This indiscriminate use of antibiotics has being a major concern for health specialist. Increased resistance to Gentamicin, Amoxicillin/ Ampicillin, Ceftazidime and Cefuroxime has been found to be due to non-investigation of children put on antibiotics, unjustified and prolonged use of antibiotics, lack of utilisation of laboratory investigations and delay in obtaining laboratory results (Musoke and Revathi, 2000).

As shown in this study cefuroxime, gentamicine, augmentin, cefixime, ceftriaxone and ampicillin which are some of the antibiotics recommended by WHO against bacteremia might gradually become less effective as they are all highly resistant to the bacteria. This will pose a great challenge to the medical practitioners because many of the other available antibiotics have not being recommended for use in children due to their metabolic differences and if the ones that are recommended are becoming less effective then antibiotic resistance will begin to play a significant role in the occurrence and severity of epidemics of disease. Plasmid has been documented to have encoded gene that provides resistance to naturally occurring antibiotics in competitive environmental niche (Lipps, 2008; Kroll *et al*., 2010). This study confirms that resistance in bacterial isolates is not only plasmid- mediated. Although, 37.5% of the isolates carried plasmid but not all are responsible for the antibiotic resistance. However, in this study, resistance in all (100%) the *Staphylococcus aureus* that showed multiple resistance was due to the presence of plasmid. furthermore, resistance in *pseudomonas aeruginosa* is not plasmid mediated as shown by the post curing sensitivity test. Resistance of bacterial not due to plasmid might be due to the efflux mechanism which has being identified in *pseudomonas aeruginosa* (Jawertz *et al*., 2007).

## CONCLUSION

The rate of resistance of bacteria associated with bacteremia is high in this study therefore a proper laboratory test should be carried out before antibiotics is accompanied by antimalaria as it was not all the children who has malaria who also have bacteremia. This will not only reduce the rate of resistance but will also help to know the true incidence of malaria and bacteremia which will help in the effective management of the health sectors.

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