Nasal colonisation of drug resistant bacteria in Ghanaian children less than five years

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Citation

E Donkor, E Nartey. *Nasal colonisation of drug resistant bacteria in Ghanaian children less than five years*. The Internet Journal of Microbiology. 2007 Volume 5 Number 2.

Abstract

Bacteria isolated from nasal specimens collected from 100 subjects less than 5 years, were identified and their susceptibility characterised by standard microbiological methods. The organisms isolated from the subjects and prevalence rates were as follows: Staphylococcus aureus (49%), coagulase negative staphylococcus (27.4%), Streptococcus pneumoniae (4%), viridian streptococci (4%), Streptococcus pyogenes (2%) Klebsiella spp. (7%) Escherichia coli (2%), Enterobacter spp. (2%), Pseudomonas spp. (2%), and Morganella morgani (1%). Sex and age were not significantly associated with bacteria carriage at $p<0.5$. The prevalence of multiple drug resistance in Staphylococcus aureus was 49%, while among the Streptococci and the gram-negative rod organisms, it was 90% and 58.3% respectively. The prevalence of resistance of Staphylococcus aureus (the predominant organism isolated) to drugs tested, showed over 50% resistance for five of the nine drugs, including, cefuroxime, ampicillin, cotrimoxazole, tetracycline, penicillin, while resistance of 15-25% were observed for gentamicin, cloxacillin, erythromycin, and cefotaxime. The nasal area of the children appears to be an important source of multiply resistant bacteria.

INTRODUCTION

Antibiotic-resistant organisms lead to increased hospitalizations, health costs, and mortality. Antimicrobial drug resistance has therefore become an important public health concern associated with serious consequences for the treatment of infections ([1,2]). The phenomenon has been attributed to the misuse of antimicrobial drugs which provide selective pressure favouring the emergence of resistant strains. One of the major interventional strategies for containing the problem of antimicrobial resistance includes effective surveillance of resistance among common pathogens such as Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria gonorrhoeae. It has become important to include normal flora in drug resistance surveillance. This is due to the escalation of resistance of normal flora recently, and the fact that bacterial normal flora could transmit drug resistance to pathogens ([3]). Additionally, though infection with bacterial pathogens usually comes from an extrinsic source, they could also be from intrinsic sources where bacterial normal flora, which normally do not cause diseases become opportunistic causes of infections ([3]). This could occur when a person is in an immunosupressed state and thus the immune system cannot mount a strong enough response to combat infections. This is usually the case of people with an underlying disease such as sickle cell disease and also people at the extremes of life such as infants ([4,5]).

Drug resistance is a global problem, however, it tends to carry more significance in the developing world, where treatment options are limited and lack of resources constrain implementation of surveillance. In Ghana, the incidence of bacterial resistance is known to be very high ([6]), probably due to the poor enforcement of drug policies on antibiotic usage. At present, information available on bacterial resistance in the country is inadequate, and is mainly related to clinical isolates, while very little is known about resistance of normal flora. Antimicrobial therapy in Ghana is empirical in majority of cases, and surveillance data on resistance from clinical and normal flora isolates is urgently needed for effective treatment.

The aim of this study was to assess the nasal carriage of drug resistant bacteria in children less than five years, and the possible relationship with age and sex.

MATERIALS AND METHODS

This prospective study was carried out at the Child Health Department of the Korle-Bu Teaching Hospital (KBTH) in 2006. This hospital is the largest hospital in Ghana and serves as a referral center with about 1600 beds ([7]). It is also

one of the largest and most well equipped teaching hospital in West Africa. Subjects for this study were both male and female children aged less than 5 years visiting the Out Patient Department of the hospital. A sample size of 100 was chosen based on 95% confidence limit with an allowable error of 10%. All children aged less than five years attending the hospital during the study period were eligible for inclusion in the study, while those who had taken antibiotics two weeks to reporting to the hospital were generally excluded. After ethical clearance and informed consent from the mothers of the study subjects, nasal swabs were taken with sterile cotton tipped swab sticks. A structured questionnaire was used to gather data on vital statistics and whether or not their wards had been given antibiotics in the last 2 weeks.

Specimens were immediately inoculated into Brain-Heart Infusion broth and transported to the laboratory. After overnight incubation at 37oC, they were inoculated on Blood Agar (BA), Chocolate Agar (CA) and MacConkey agar for isolation and identification of organisms. The inoculated plates were incubated at 37oC for 18 to 24 hours and re-incubated for 48 hours when no bacterial growth was noted after 18 hours incubation. CA plates were incubated at 37oC in 5% carbon-dioxide overnight (18-24 hours). Colonial morphology of organisms were noted and confirmed with Gram Staining of slides. Isolates were identified by standard biochemical methods ($_{8}$). Analytical profile index (API) was used to confirm a number of the identified organisms. The Kirby Bauer method ($_{9}$) of sensitivity testing was employed to determine the antibiogram of the isolates using the following antimicrobial drugs with their disk content in microgram (ug):- ampicillin ($_{10}$), tetracycline (^{30}), cotrimoxazole ($_{25}$), gentamicin (^{30}), cefuroxime (^{30}), cefotaxime (^{30}), penicillin ($_{10}$), erythromycin ($_{15}$), cloxacillin ($_{5}$), and, all commonly used in Ghana.

RESULTS

Organisms isolated from the study subjects included a wide range of bacteria as shown in Table 1. The most prevalent organism was Staphylococcus aureus (49%). This organism together with coagulase negative staphylococci (27.4 %) was isolated from 76 % of the study subjects. Very few numbers of other organisms were isolated, and these were mainly streptococci and gram negative rods. The streptococci, comprising Streptococcus pyogenes (2%), Streptococcus pneumoniae (4%), and viridian streptococci (4%) were isolated from 10% of the children. The gram negative rods, comprising Klebsiella spp. (7%), Escherichia coli (2%), Enterobacter spp. (2%), and Pseudomonas spp. (2%) were isolated from 13% of the children. There was no significant association of nasal bacterial colonization with sex or age at $p<0.5$.

Figure 1

Table 1: Prevalence of bacterial agents among study subjects

Bacterial agent	Prevalence
Staphylococcus aureus	49%
Coagulase negative staphylococcus	29%
Streptococcus pneumoniae	4%
Streptococcus pyogenes	2%
Viridan streptococcus	4%
Klebsiella spp.	7%
Escherichia coli	2%
Enterobacter spp.	2%
Pseudomonas spp.	1%
Morganella morgani	1%

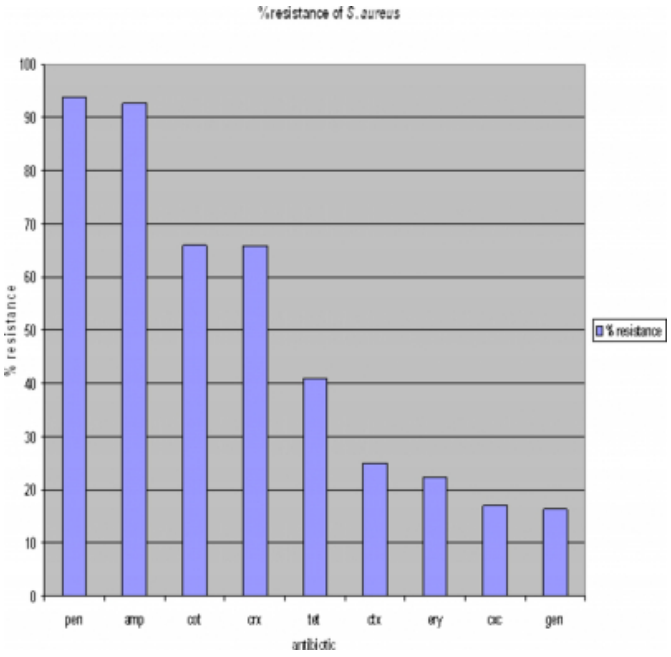
The prevalence of multiple drug resistance in Staphylococcus aureus was 49%. Among the Streptococci, the prevalence of multiple drug resistance was 90%, while among the gram-negative rod organisms, it was 58.3%. Among the various drugs, prevalence of resistance for all organisms was similar to that of Staphylococcus aureus, the main organism isolated, and is shown in Figure 1. A percentage resistance of over 50% was observed for five of the nine drugs tested, including, cefuroxime, ampicillin, cotrimoxazole, tetracycline, penicillin, while resistance of 15-25% were observed for gentamicin, cloxacillin, erythromycin, and cefotaxime.

DISCUSSION

The nasal specimens yielded a wide range of bacterial agents with Staphylococcus aureus as the principal organism at a prevalence rate of 49%. Nasal Staphylococcus aureus prevalence of 48%, 32.4%, 23.4%, 30.5% and 50.0% have been reported in Pakistan ($_{10}$), United States ($_{11}$), Malaysia ($_{12}$), Italy ($_{13}$) and Nigeria ($_{14}$) respectively. However, these studies were not carried out on children but mostly on the general population. Lamaro-Cardoso et al. (2007) reported a prevalence rate of 13.7% in Brazilian children less than five years ($_{15}$). However, a very recent study in Brazil shows that the carriage rate could be as high as 48.7% ($_{16}$), which is similar to the results of our study.

Figure 2

Figure 1: Percentage resistance of to various antimicrobial drugs



Carriage rates of 17.9%, 18.7%, and 28.4% have also been reported in children in other parts of the world ([17,18,19]).

There was no significant association of nasal bacterial colonisation with age and sex, though some previous reports had shown this relationship for age ([20]). The absence of this observation in the study may be due to the small age range of less than five years used.

Resistance of normal flora is of concern, and in this study, the prevalence of multiple drug resistance of nasal flora Staphylococcus aureus was 49% which is quite high. A recent study with clinical isolates of Staphylococcus aureus in Ghana had showed a prevalence of 80% ([21]). This shows a high trend of resistance for both clinical and normal flora Staphylococcus aureus isolates. Nasal carriage of multiply resistant Staphylococcus aureus is of serious concern because it is a risk factor for infection ([22]). Since the study was done in a hospital environment, an issue of concern is the spread of these resistant isolates in nosocomial infections.

High levels of resistance were observed for penicillin, ampicillin, and cotrimoxazole. These are drugs which had been reported as having high percentage resistance for a lot of microorganisms for several years, and the rate of resistance had been rising over the years not only for clinical isolates but also for the normal intestinal flora of the healthy population ([23,24]). Lower prevalence rates of resistance were observed for gentamicin, cloxacillin, erythromycin and cefotaxime. Relatively these drugs have been on the Ghanaian market for a relatively short period of time as compared to drugs like ampicillin, and therefore may not have been subjected to high use and or misuse. In addition, some of these drugs are expensive and may be prescribed for serious infections, thus limiting their usage.

Extremely high resistance rates were observed for Staphylococcus aureus to penicillin and ampicillin. The excessively high resistance to these drugs is probably due to β-lactamase production by the organism, which degrades the β-lactam ring of these drugs. In this study, methicillin resistance of Staphylococcus aureus was inferred from cloxacillin resistance ([25]). The prevalence of methicillin resistant Staphylococcus aureus was 15% which is similar to the prevalence rates observed in studies in some developed and developing countries ([26,27]). High prevalence rates such as 42.9% as well as low rates of 0.33%, 1.02%, 2.2% and 2.7% have also been documented ([15,16,17,18,19]).

Susceptibility tests were not carried out on coagulase negative Staphylococci. This is because the organism is usually not pathogenic to older children ([4]). Owing to the few members of the other organisms isolated, it is difficult to comment adequately on their antimicrobial susceptibility results. However multiple drug resistance of about 90% and 58% observed for the gram-negative rods and Streptococci respectively probably indicate widespread resistance among these organisms.

The study has shown that the nasal area of the children habour various multiply resistant bacteria. This is of public health concern, given the fact that bacterial normal flora could transmit drug resistance to pathogens, and could also cause infections in people with lowered immunity such as infants who formed the subjects in this study.

ACKNOWLEDGEMENTS

The technical support provided by the entire staff of the Department of Microbiology, University of Ghana Medical School is gratefully acknowledged.

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