ORIGINAL ARTICLE

ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF BACTERIA ASSOCIATED WITH UPPER RESPIRATORY TRACT INFECTIONS IN KITUI, KENYA

Mutinda Josphat, MD1* Maingi John, MD,2 Kebira Anthony, MD3

ABSTRACT

Background: This study aimed at determining the prevalence of bacterial agents causing upper respiratory tract infections and their susceptibility patterns to commonly used antibiotics among outpatients in Kitui District Hospital.

Methods: A total of 237 throat swabs were collected between November, 2012 to April, 2013 and inoculated onto Blood agar, MacConkey agar and Chocolate agar then incubated at 37 °C for 24 hours. Colony morphology and standard biochemical tests were performed for identification and confirmation of the isolates based on their Gram staining and cultural characteristics. Antimicrobial sensitivity patterns of the bacteria to antibiotics were determined by Kirby-Bauer disc diffusion technique. P values of ≤ 0.05 were considered to have clinical and epidemiological significance.

Results: Pathogens were isolated in 95.4% of the samples collected, out of this, 5% were mixed cultures involving Candida albicans and either viridans group streptococci or Staphylococcus aureus. Bacteria isolated were S. aureus with the highest prevalence (44.3%), followed by viridans group streptococci (32.5%) and Streptococcus pyogenes (13.5%). Resistance of bacterial pathogens to antibiotics in Kitui district hospital was highest in viridans group streptococci (48.2%) followed by Staphylococcus aureus (40.5%) while Streptococcus pyogenes had the least resistance (28.1%) and there were no cases of multi-drug resistance.

Conclusion: The rate of antibiotic resistance was significantly high and proper interventions should be put in place by the relevant government ministry to prevent these infections and their complications.

Key words: Prevalence, susceptibility, antibiotic resistance, multi-drug resistance, URTIs

INTRODUCTION

It has been widely acclaimed that the respiratory tract is the most frequent site of infection as it comes into direct contact with the physical environment and is exposed to airborne microorganisms such as viruses, bacteria, fungi and parasites (1). Beta-hemolytic streptococci, Corynebacterium diphtheriae, Neisseria spp, Arcanobacterium haemolyticum, Chlamydia pneumoniae, Mycoplasma pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae, Bordetella pertussis and Moraxella catarrhalis are the most common bacteria associated with URTIs (2). These pathogens invade the mucosal lining of the upper respiratory tract leading to a disease condition (3). The incubation period of most URTIs last from a few hours to 3 days after exposure while the symptoms may last for 7-10 days or even longer (4). Proper judgment is required in differential diagnosis of URTIs since there are several related conditions that may have similar or overlapping clinical signs and symptoms (4).

Various studies have shown that antibiotic use improves the health of patients with upper respiratory tract infections (5,6). Moreover, antibiotics seem to increase cure rates and reduce duration of some upper respiratory tract infections whose microbiological diagnosis suggest bacterial etiology (7). However, the quality and necessity of antibiotic therapy has been questioned with various studies confirming that over 50% of antibiotic use in treatment of upper respiratory tract infections is unnecessary and may not improve the clinical outcomes (4,8). Self prescription of antibiotics often lead to increased resistance to commonly used antibiotics by bacterial pathogens, worsen the clinical outcomes and lead to high treatment cost (9). This study was carried out to determine the prevalence of URTIs...
and antimicrobial susceptibility patterns of bacterial pathogens causing upper respiratory tract infections to commonly prescribed antibiotics among patients visiting Kitui district hospital, Kenya.

**PATIENTS AND METHODS**

**Study area:** The study was carried out in Kitui District Hospital which is the largest and the busiest health facility in Kitui County, Kenya.

**Study population:** The study targeted outpatients of all ages and sexes visiting Kitui district hospital with upper respiratory tract infections from November, 2012 to April, 2013. The criteria for inclusion in the study were that the patient must be suffering from upper respiratory tract infections, must not have taken antibiotics of any kind for at least one week before the clinical visit and must consent voluntarily to participate in the study. Young children aged below 1 year, those who had taken antibiotics within one week before the clinical visit and inpatients were excluded from the study. The study was a cross-sectional study and samples were collected from 237 outpatients visiting the hospital.

**Sample collection, isolation and identification of pathogens:** Clinical samples of throat swabs were collected using sterile swabs and transported to the hospital’s bacteriology laboratory using Amies transport medium for bacteriological analysis. A loopful of each sample was then inoculated onto sterile Blood agar, Chocolate agar, sabouroud’s agar and MacConkey agar immediately on arrival and then incubated aerobically at 37°C for 24 hours except for Chocolate agar which was incubated under anaerobic conditions. The isolates were identified using standard biochemical test based on their colony morphology, colour, haemolysis on blood agar and Gram staining characteristics (10).

**Antibiotic susceptibility testing:** All bacterial isolates were subjected to *in vitro* sensitivity tests for the following antibiotics amoxiclav (30mcg), Ceftriaxone (30mcg), piperacillin (100mcg), gentamicin (10 mcg), amikacin (30mcg), meropenem (10mcg), tazobactam (10mcg) and ofloxacin (10 mcg). This was carried out using Kirby-Bauer method of antibiotic susceptibility testing (11,12).

Briefly a small inoculum of the isolated bacteria was emulsified in 3 ml sterile normal saline in bijou bottles according to 0.5 McFarland’s standard. The bacterial inoculum was inoculated in Muller-Hinton agar to form a lawn of bacteria under sterile conditions. A paper disc impregnated with the specific concentration of the antibiotics was then placed on the surface of the inoculated bacteria and incubated at 37 °C for 24 hours. Diameters of formed zones of inhibition were measured in millimetres and compared with recorded diameters of the control organisms. *Staphylococcus aureus* ATCC 25923 was used as a control for Gram positive bacteria to determine the breaking-even points of susceptibility or resistance (12).

**Data analysis:** All data was analyzed with SPSS, version 20.0 statistical software (SPSS Inc. Chicago III, USA). Chi-square test was used to compare the percentages.

**Ethical consideration:** The research protocol was approved by the ethics review committee at Kenyatta University and research permit obtained from National Commission for Science, Technology and Innovation. Informed consent was solicited from the patients through the hospital management.

**RESULTS**

**Prevalence of different pathogens:** Pathogens were isolated in 95.4% of the subjects screened. The isolated pathogens were *Staphylococcus aureus* (44.3%), viridans group streptococci (32.5%) and *Streptococcus pyogenes* (13.5%) in that order of ranking. Co-infections were found in 5 % of the patients consisting of 2.5 % co-infections of viridans group streptococci and *Candida albicans* and 2.5 % co-infections of *Staphylococcus aureus* and *Candida albicans* while there was no growth in 4.7 % of the samples. The difference in the types of pathogens associated with URTIs in different age groups was not statistically significant (P=0.129) however, majority of the pathogens were isolated from females (P = 0.0001) as depicted in Table 2.

**Sensitivity of pathogens to antibiotics:** The antibacterial susceptibility profile of the bacterial isolates revealed high level sensitivity as susceptibility of bacterial isolates to any antibacterial agent was ≥ 77.7% as depicted in table 3. The overall resistance rates were generally low for meropenem, ceftriaxone, ofloxacin and amikacin respectively. However, tazobactam had the highest resistance. The susceptibility profiles displayed by these isolates to various antibiotics followed similar patterns (P = 0.564). Generally, 58.4 % of all isolates were sensitive to all antibiotics while 41.6 % were resistant to at least one antibiotic. In these findings, trends of resistance of the isolates to several antibiotics followed similar patterns (P = 0.100).
Table 1: Prevalence of isolates in different age groups and sexes, Kitui District Hospital, Kenya, Nov. 2012– Apr. 2013

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Proportion of isolates - n (%)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>VGS SP</td>
<td>SA VGS/CASA/CA</td>
</tr>
<tr>
<td>1 – 5</td>
<td>33 (13.9)</td>
<td>12 (5.1)</td>
</tr>
<tr>
<td>6 – 14</td>
<td>7 (3)</td>
<td>3 (1.3)</td>
</tr>
<tr>
<td>15 – 25</td>
<td>10 (4.2)</td>
<td>3 (1.3)</td>
</tr>
<tr>
<td>26 – 35</td>
<td>14 (5.9)</td>
<td>7 (3.0)</td>
</tr>
<tr>
<td>36 – 45</td>
<td>6 (2.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>46 – 55</td>
<td>5 (2.1)</td>
<td>3 (1.3)</td>
</tr>
<tr>
<td>56 – 65</td>
<td>0 (0.0)</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>Above 65</td>
<td>2 (0.8)</td>
<td>2 (0.8)</td>
</tr>
</tbody>
</table>

Sex

| | VGS SP | SA VGS/CASA/CA | Total (n) |        |
|--------------------------------|---------|---------------|----------|
| Male                        | 30 (12.7) | 21 (8.9) | 29 (12.2) | 2 (0.8) | 1 (0.4) | 83 (36.7) | 0.0001 |
| Female                      | 47 (19.8) | 11 (4.6) | 76 (32.1) | 4 (1.7) | 5 (2.1) | 143 (63.3) |        |
| Total (n)                   | 77 (32.5) | 32 (13.5) | 105 (44.3) | 6 (2.5) | 6 (2.5) | 226 (95.3) |        |

Key: (VGS – Viridans group streptococci, SP – S. pyogenes, SA – S. aureus, CA – C. Albicans)
P- values were obtained using Chi-square test

Table 2. Sensitivity of isolates to antibiotics, Kitui District Hospital, Kenya, Nov. 2012– Apr. 2013

<table>
<thead>
<tr>
<th>Sensitivity of isolates (%)</th>
<th>VGS (n=83)</th>
<th>S. pyogenes (n=32)</th>
<th>S. aureus (n=111)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>I</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Amoxiclav</td>
<td>68.9</td>
<td>12.3</td>
<td>18.8</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>88.2</td>
<td>3.4</td>
<td>8.4</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>77.7</td>
<td>4.2</td>
<td>18.1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>82.6</td>
<td>5.3</td>
<td>12.1</td>
</tr>
<tr>
<td>Amikacin</td>
<td>91.3</td>
<td>3.7</td>
<td>6.0</td>
</tr>
<tr>
<td>Meropenem</td>
<td>98.2</td>
<td>1.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Tazobactam</td>
<td>63.1</td>
<td>3.2</td>
<td>33.7</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>97.9</td>
<td>2.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Resistance to Antibiotics</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive to all</td>
<td>51.8</td>
</tr>
<tr>
<td>Resistant to ≤ 3</td>
<td>48.2</td>
</tr>
<tr>
<td>Resistant to ≥ 4</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Key: S – Sensitive, R – Resistant, I – Intermediate
P- Values were obtained using Chi-square test

DISCUSSION

Isolates of S. aureus, S. pyogenes, viridans group streptococci and C. albicans represent clinically significant pathogens. These pathogens have also been associated with RTIs in other studies (13-16). Isolation of S. pyogenes was within the range reported in most studies. A similar study reported a prevalence of 14.7% of this pathogen in Brazil, although the pathogen was found to be predominant in children between 3–12 years which is contrary to these findings (17). It was also reported with a prevalence of 22% in Iceland (18), 20.1% in Buea, Cameroon (19) and 8% in Netherlands (20). Previous studies have emphasized the importance of establishing the appropriate treatment for this infection to reduce chances of complications such as rheumatic fever, endocarditis and scarlet fever (21-23). The high prevalence of S. aureus has also been reported in United States (24) and Europe (25), respectively. Another study reported a prevalence
rate of 21.6% in Europe (26), 23.4% in Malaysia (27) and 26% in Thirroul, New South Wales (28).

Co-infections were also observed in this study. A similar study reported a prevalence of 13% of Candida Spp. in Mbagathi district hospital, Nairobi, Kenya (29). The higher risk of contracting these infections in children below 5 years of age has also been reported in other studies (30-32) which may be as a result of a weak immune system which is not fully developed while in adolescents and young adults, it could be caused by hormonal imbalances which may affect the immune system thus predisposing them to infections (33,34). However, sex has no influence on these infections. Imbalance of the oral microbiota as a result of Broad-spectrum antibiotics which eliminate the competing bacteria and disrupt the normally balanced ecology of oral micro-organisms or medication with corticosteroids may also predispose patients to these infections (35).

It is possible for candidiasis to spread from the mouth to other sites such as the pharynx, oesophagus, lungs, liver, ano-genital region, skin or even the nails especially in debilitated individuals (36,37) and this may explain the isolation of this pathogen from the oropharynx. The role of oral thrush in the hospital and ventilated patients has not been clearly described; however it has been proposed that, there is a risk of positive interaction of Candida spp. with topical bacteria (37). This interaction could be associated with increased risk of ventilator associated pneumonia (38) and therefore necessitates the establishment of proper treatment of these infections to reduce such risks. The Candida load in the mouth which is thought to be the source of these infections can also be reduced by improving oral hygiene measures such as regular brushing of teeth and use of antimicrobial mouthwashes (35).

More pathogens were isolated from females than males and this was statistically significant; however, this could be strongly associated with the composition of the sample which consisted of more females than males. Failure to isolate pathogens in 4.7% of the samples could be attributed to the patients' use of antibiotics prior to visiting the hospital or viral etiology of these infections. Furthermore, other pathogens such as H. influenzae, S. pneumoniae, K. pneumoniae and P. aeruginosa which have been incriminated for causing these infections in other studies (39-42) were not isolated. Although it may not be possible to completely explain this phenomenon in the present study, it can be suspected that the patients had taken antibiotics although the inclusion criteria excluded patients who were on antibiotics one week prior to sampling or the infections were associated with viral etiology.

Resistance of viridans group streptococci was the highest as observed in this study, this is important particularly considering that these bacteria have been considered to be part of the normal flora. Resistance of this pathogens has also been reported in other studies (43-45). Brook (16) reported that these could be a source of infection of upper respiratory tract. It has further been shown that this resistance could be passed on to other sensitive pathogens through sharing of resistance markers (45).

The resistance of isolates of Streptococcus pyogenes has been observed in other studies(1). Previously, resistance of this pathogen to penicillin has also been reported in other studies (46-48). Internalization and intracellular survival of this pathogen has been suggested as an explanation to this resistance (47).

There was no antibiotic to which Staphylococcus aureus demonstrated 100% sensitivity although the resistance was generally low. This low rate of antibiotic resistance by S. aureus was also been reported across nine European countries (26). This pathogen has been reported to be a β-lactamase producer which has the ability to cause therapeutic failures by mediating antibiotic resistance. The occurrence of bacterial resistance mediated by β-lactamases has been reported in Nigeria and South Africa (49-51). A similar study in Vietnam reported that 90 % of S. aureus were resistant to at least one antibiotic (39). In Nigeria, it was reported that 87.7 % of S. aureus were resistant to at least one antibiotic with 41.2 % being multi-drug resistant (1). However in this study, only 40.5 % of S. aureus were resistant to at least one antibiotic and there was no case of multi-drug resistance since no organism was resistant to more than three antibiotics.

There was no statistically significant difference in the trends of resistance of isolates to several antibiotics; however 41.5% of all isolates were resistant to at least one antibiotic. This may be as a result of the choice of antibiotics selected for sensitivity testing in this study because all of them are normally preserved for 2nd or 3rd line of treatment for complicated bacterial infections (52-54).

Conclusion: Pathogens isolated were S. aureus, S. pyogenes, viridans group streptococci and C. albicans. S. aureus was the most prevalent pathogen in this study, followed by viridans group streptococci.
while *S. pyogenes* was the least encountered bacteria. The prevalence of these isolates was significantly different.

Majority of the pathogens were isolated in patients aged between 1–5 years. Notably, no pathogen was significantly more prevalent than the others in this age group. Patients aged between 6–14 and 15 -25 are at a higher risk of getting mixed infections of *C. albicans* and viridans streptococci compared to those in other age groups.*S. pyogenes* was more prevalent in males than in females while *S aureus* was more prevalent in females than in males. Based on these findings, it can be conclusively reported that the prevalence of different types of bacteria associated with upper respiratory tract infections is not uniform in all the age groups and sexes.

Viridans streptococci were more sensitive to gentamicin and ofloxacin and less sensitive to piperacillin and tazobactam compared with the other isolates. Isolates of *S. aureus* were more sensitive to piperacillin and tazobactam and less sensitive to gentamicin and ofloxacin compared to the other isolates. Sensitivity of *S. pyogenes* was within the range of the others for all antibiotics tested. Resistance of bacterial pathogens to at least one antibiotic was highest in viridans group streptococci, followed by *S. aureus* while *S. pyogenes* had the least resistance among the isolates and there was no case of multi-drug resistance.

**Recommendations:** Proper interventions should be put in place to prevent young children aged between 1 and 5 years from contracting and transmitting upper respiratory tract infections because they are at a higher risk of getting these infections than the other age groups we also recommend continuous surveillance to monitor the prevailing pathogens associated with upper respiratory tract infections and their antimicrobial susceptibility patterns from time to time. This study further recommends that, more studies to be done to determine the prevalence of other pathogens such as viruses and fungi that are associated with these infections and their interactions with bacterial pathogens. More studies need to be carried out to assess the rate of asymptomatic carriage of these pathogens in the upper respiratory tract of healthy persons since this is a major source infection by these pathogens.

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