INTRODUCTION

Antibiotic resistance is growing worldwide and its impact is more in poor countries where infections are high due to poverty, unhygienic environment and poor health facilities. In such settings, the morbidity and mortality rate is also higher due to infectious diseases. The challenge of resistance also persists in developed countries. There is also emergence of multidrug resistant organisms which have made the situation worse. *Staphylococcus Aureus*, a facultative anaerobic gram positive coccus, is a part of skin flora and causes skin infections such as cellulitis, scalded skin syndrome as well as life threatening conditions like meningitis, Pneumonia, Endocarditis and Sepsis. It is best known to be the cause of nosocomial infections. Antibiotics are life saving drugs and it must be remembered that not only the organism but the excessive unnecessary use of these life saving drugs is also a major cause.
of resistance. *Staphylococcus aureus* has developed multiple ways to become resistant to many antibiotics. This involves production of beta lactamase enzymes, changes in cell wall structure and genetic mutations. The organism also has one of the most dangerous strains known as Methicillin Resistant *Staphylococcus aureus* (MRSA) which are resistant to most of the antibiotics except Glycopeptide group e.g. Vancomycin and Teicoplanin. But resistance has been reported against Vancomycin and Teicoplanin as well, therefore there is a need for a surveillance program in every country to assess the level of resistance. In Europe, Antimicrobial Resistance Surveillance System (EARSS) is a surveillance network which works in 31 countries and consists of over 400 laboratories. It reports antimicrobials resistance patterns every 18 months from Europe. In Pakistan, antibiotics are sold over the counter without any prescription, moreover culture and sensitivity is also not routinely done and physicians and chemist are using the drugs without thinking about long term consequences like resistance. There is no surveillance network to check the prescriptions and to compile reports from laboratories to determine the antibiotic resistance patterns of different organisms. Health education and schools of hygiene are very limited and so is the number of studies done till now to report the antibiotic resistance in the Khyber Pakhtunkhwa Province. Therefore, we selected a major tertiary care hospital of Peshawar for this purpose to report for the first time the pattern of resistance and sensitivity found here. The results of this study will help us to know the current situation and would also give us an opportunity to attract researchers to find the causes of increased resistance.

**METHODOLOGY**

This was a retrospective study over a period of one year i-e from October 2010 to October 2011. During this period, a total of 2058 clinical isolates were received for culture and sensitivity at the Pathology Laboratory of Lady Reading Hospital Peshawar. The samples were collected from pus, blood, urine, wound sites and other body fluids using sterile techniques. Purposive sampling was done. All samples received for culture and sensitivity were included in the study. Samples which did not yield *Staphylococcus aureus* growth were excluded altogether. Out of 2058 samples, growth of *Staphylococcus aureus* was obtained in 723 samples. Of these 723 samples, 699 were from pus, 16 from blood and 8 from urine. Incubation of the samples was done on Mannitol Salt agar, McConkey’s agar and Sheep blood agar at 37°C for 24 hours. CLED agar plates were used for culture of urine samples. Colony morphology, Gram staining using crystal violet dye, catalase test and coagulase test was used to identify *Staphylococcus aureus*. Following revised 2010 Clinical and Laboratory Standard Institute guidelines, antibiotic susceptibility testing using Kirby Bauer’s disc diffusion technique and interpretation of inhibition zone diameters (in mm) was done. The growth was subjected to a total of 14 drugs from five different classes of antibiotics to check the sensitivity pattern of the *Staphylococcus aureus*. Data entry and analysis was done using the Statistical Package for the Social Science SPSS 16.0. Univariate analysis was done using the Statistical Package for the Social Science SPSS 16.0. Univariate analysis was done using the Statistical Package for the Social Science SPSS 16.0. Univariate analysis was done using the Statistical Package for the Social Science SPSS 16.0. Univariate analysis was done using the Statistical Package for the Social Science SPSS 16.0. Univariate analysis was done using the Statistical Package for the Social Science SPSS 16.0. Univariate analysis was done using the Statistical Package for the Social Science SPSS 16.0.

**RESULTS**

The results of the study are given in Table 1 and 2.

Table 1 shows the number and type of those isolates in which the growth of *Staphylococcus aureus* was obtained. Resistance and sensitivity of 14 antibiotics have been shown in Table 2. Only generic names of antibiotics have been used and the class to which the drug belongs.
We found 228 (31.5%) cases of MRSA (Methicillin Resistant Staphylococcus aureus) during the 12 months study. Our results differ from studies conducted elsewhere in Pakistan because the incidence varies a lot among hospitals but incidence is mostly reported higher in big cities. In a multicentre study conducted on 792 clinical isolates of Staphylococcus aureus from 8 laboratories all over Pakistan i.e. Karachi, Peshawar, Lahore, Sukkur, Islamabad, Quetta, and Mirpur, Azad Kashmir, the incidence of MRSA was found to be 2-61% while specifically in Peshawar it was 36%. In another study conducted in Karachi from January 2009 to December 2009, there were 174 (38.6%) MRSA cases out of a total of 450 isolates. It is worth mentioning that it also reported one case of VRSA (Vancomycin Resistant Staphylococcus aureus). In another study conducted from December 2009 to January 2008 in CMH Pano Akil, only 19.8% cases were that of MRSA. We did not find any strain of Staphylococcus aureus resistant to Vancomycin making it the only antibiotic to which all strains were susceptible and this has been found out in other studies also. We did encounter higher i.e. 19.8% resistance to Teicoplanin and further research, in isolating such resistant strains of Staphylococcus aureus as well as investigating the mechanism of resistance of these strains to Teicoplanin, should certainly be done. Teicoplanin has otherwise very good activity against Staphylococcus aureus as seen in other studies in Pakistan.

Table 2: antibiotic drug resistance and sensitivity in Staphylococcus aureus isolates

<table>
<thead>
<tr>
<th>S No.</th>
<th>Class of Antibiotic</th>
<th>Generic name of Antibiotic</th>
<th><em>resistant</em></th>
<th>Sensitive</th>
<th>Drug tested on (number of samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Penicillin Group</td>
<td>Methicillin</td>
<td>228 31.5</td>
<td>495 68.5</td>
<td>723</td>
</tr>
<tr>
<td>2.</td>
<td>Penicillin Group</td>
<td>Amoxicillin</td>
<td>713 98.6</td>
<td>10 1.4</td>
<td>723</td>
</tr>
<tr>
<td>3.</td>
<td>Penicillin Group</td>
<td>Amoxicillin + Clavulanic Acid</td>
<td>330 45.6</td>
<td>393 54.4</td>
<td>723</td>
</tr>
<tr>
<td>4.</td>
<td>Penicillin Group</td>
<td>Piperacillin + Tazobactam</td>
<td>57 34.6</td>
<td>108 65.4</td>
<td>165</td>
</tr>
<tr>
<td>5.</td>
<td>Glycopeptide</td>
<td>Vancomycin</td>
<td>0 0.0</td>
<td>723 100</td>
<td>723</td>
</tr>
<tr>
<td>6.</td>
<td>Glycopeptide</td>
<td>Teicoplanin</td>
<td>143 19.8</td>
<td>580 80.2</td>
<td>723</td>
</tr>
<tr>
<td>7.</td>
<td>Carbapenem</td>
<td>Meropenem</td>
<td>296 40.9</td>
<td>427 59.1</td>
<td>723</td>
</tr>
<tr>
<td>8.</td>
<td>Carbapenem</td>
<td>Imipenem + Cilastatin Sodium</td>
<td>304 42.0</td>
<td>419 58.0</td>
<td>723</td>
</tr>
<tr>
<td>9.</td>
<td>Cephalosporin</td>
<td>Ceftriaxone</td>
<td>365 50.5</td>
<td>358 49.5</td>
<td>723</td>
</tr>
<tr>
<td>10.</td>
<td>Cephalosporin</td>
<td>Ceftazidime</td>
<td>148 98.0</td>
<td>3 2.0</td>
<td>151</td>
</tr>
<tr>
<td>11.</td>
<td>Cephalosporin</td>
<td>Cefoperazone</td>
<td>143 94.7</td>
<td>8 5.3</td>
<td>151</td>
</tr>
<tr>
<td>12.</td>
<td>Cephalosporin</td>
<td>Cefoperazone</td>
<td>374 51.7</td>
<td>349 48.3</td>
<td>723</td>
</tr>
<tr>
<td>13.</td>
<td>Fluoroquinolone</td>
<td>Ciprofloxacin</td>
<td>111 73.5</td>
<td>40 26.5</td>
<td>151</td>
</tr>
</tbody>
</table>

has been mentioned.

DISCUSSION

We found 228 (31.5%) cases of MRSA (Methicillin Resistant Staphylococcus aureus) during the 12 months study. Our results differ from studies conducted elsewhere in Pakistan because the incidence varies a lot among hospitals but incidence is mostly reported higher in big cities. In a multicentre study conducted on 792 clinical isolates of Staphylococcus aureus from 8 laboratories all over Pakistan i.e. Karachi, Peshawar, Lahore, Sukkur, Islamabad, Quetta, and Mirpur, Azad Kashmir, the incidence of MRSA was found to be 2-61% while specifically in Peshawar it was 36%. In another study conducted in Karachi from January 2009 to December 2009, there were 174 (38.6%) MRSA cases out of a total of 450 isolates. It is worth mentioning that it also reported one case of VRSA (Vancomycin Resistant Staphylococcus aureus). In another study conducted from December 2009 to January 2008 in CMH Pano Akil, only 19.8% cases were that of MRSA. We did not find any strain of Staphylococcus aureus resistant to Vancomycin making it the only antibiotic to which all strains were susceptible and this has been found out in other studies also. We did encounter higher i.e. 19.8% resistance to Teicoplanin and further research, in isolating such resistant strains of Staphylococcus aureus as well as investigating the mechanism of resistance of these strains to Teicoplanin, should certainly be done. Teicoplanin has otherwise very good activity against Staphylococcus aureus as seen in other studies in Pakistan. In a study conducted in Islamabad during the period from December...
2007 to August 2008, only 3% resistance against Teicoplanin was found. Tertiary Hospitals should take more steps to ensure hygiene and decrease the burden of these deadly strains.

Our study shows that *Staphylococcus aureus* has less sensitivity to Penicillin group of antibiotics. We found that only 1.4% organisms were susceptible to Amoxicillin. With the addition of Clavulanic acid to Amoxicillin, the sensitivity increased from 1.4% to 54.4%. Therefore, it suggests that a great number of strains produce Beta lactamases enzymes and combination of Amoxicillin with Clavulanic acid is much better. The combination of Piperacillin with Tazobactam was also a better combination (65.4% sensitivity) and it was found more effective than Amoxicillin + Clavulanic Acid. It must be noted that in many studies like this, resistance to Beta-lactam Antibiotics was found higher than other groups. In a multicentre study in Pakistan, during the period from April 2006 to March 2008, resistance to Oxacillin, Penicillin and Ampicillin was found to be 100% while that of Cephalothin was 92.4%.

Meropenem and Imipenem+Cilastatin Sodium did not differ much in their activity against *Staphylococcus aureus* with 59.1% and 58.0% sensitivity respectively. These antibiotics are resistant to degradation by beta lactamases and that is why their sensitivity is more than Amoxicillin + Clavulanic Acid. Imipenem + Cilastatin Sodium, has been shown in studies of Japan and USA, as very active against *Staphylococcus aureus*.

Cephradine – a first generation Cephalosporin has very good activity against gram positive organisms. We found 46.2% resistance to Cephradine making it superior than the third generation Cephalosporins and Fluoroquinolones used in our study but inferior to Carbapenems. Resistance against Cefoperazone and Ceftazidime has come very high in our study. Studies have shown that if Cephalosporins are combined with Beta lactamases inhibitors, their efficacy can be greatly increased.

Although Fluoroquinolones are not considered the first line drugs for gram positive organisms yet they have a broad spectrum and Ciprofloxacin, the earlier and the most common drug used of this group in our hospitals, had excellent activity against *Staphylococcus aureus* including Methicillin resistant strains when it was introduced but Topoisomerase and other genetic mutations have made *Staphylococcus aureus* resistant to these old drugs – Ciprofloxacin and Enoxacin. We encountered 51.7% resistance to Ciprofloxacin. A study from Karachi reported 63.7% resistance to Ciprofloxacin in 2009. The resistance to Enoxacin was very high 73.5%, but it is not unusual as Enoxacin is less effective than Ciprofloxacin. Studies have reported that the fourth generation Fluoroquinolones like Moxifloxacin have little resistance but it is not widely used here and therefore not tested.

**CONCLUSION**

Incidence of MRSA was 31.5% which is very high. We conclude that except 3 antibiotics i.e. Vancomycin, Teicoplanin and Methicillin, the resistance to all the other antibiotics was more than 40%. Moreover, the resistance to Amoxicillin, Cefoperazone and Ceftazidime is more than 90% and should not be prescribed for such infections. Guidelines regarding the use of antibiotics must be made and strict laws also implemented to stop the free use of these drugs by quacks and chemists also. The hospitals should improve their hygiene to avoid spread of these dangerous organisms. National guidelines would ideally be based on several larger comprehensive studies and the authors recommend such studies be done especially in Khyber Pakhtunkhwa involving other hospitals also. Education of doctors is very necessary now as new pattern of resistance and sensitivity require them to know the effective drugs for each organism and do not prescribe unnecessary and ineffective antibiotics. If this is not done, it is very likely that in future these life saving drugs would be useless as a result of increased resistance.

**REFERENCES**

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CONTRIBUTORS

MN Supervised the whole project, revised the article and gave the final approval. MA wrote the article for the first time, did the data analysis, literature review and edited the article. SMN took part in literature review, data analysis and interpretation. SHA, MZUIK & AK took part in study design, data collection, entry and revision of the article in every stage. MUK reviewed the article in every stage and helped a lot in study design, literature review and final revision of the article. The project was self funded. All authors have taken part in its writing, interpretation of data revision and all authors approve its final version.