Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Pakistan

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Abstract

Background: Urinary tract infection (UTI) is a common infection in Pakistan. Poor knowledge about personal hygiene and lack of awareness are major reasons of this infection. All over the world and in Pakistan E. coli and K. pneumonia are main causative agents of UTI.

Objectives: To analyze antibiotic susceptibility pattern of bacterial strains isolated from urinary tract infections.

Methods: In present study total 227 samples from Genomic Research Lab and Diagnostic Center Rawalpindi, Pakistan were tested for presence of different microbial infectious agents and their antibiotic resistance and susceptibility pattern. Biochemical test such as Gram staining, Lactose fermentation test, Motility test, Catalase test and Colony formation on MacConkey agar, Indole test, and Citrate utilization test were applied. The antibiotic susceptibility and resistance pattern was evaluated by using fourteen different antibiotics on MullarHigton agar. Top ten antibiotics were selected at a cut off value of 100 samples for E. coli and 20 samples for Klebsiella.

Results: Against E. coli and K. species the most susceptible antibiotic was Imipenam with 97.5% and Amikacin with 94.9% susceptibility respectively. Moreover, against E. coli and K. species the most resistance antibiotic was Ciprofloxacin 81.7% and Tobramycin with 76.1% resistance respectively. The comparison of present study with previous studies had shown an increase in resistance against various antibiotics.

Conclusion: It is concluded from the present study that antibiotic resistance is on raise in Pakistan and the effect of each antibiotic was different with respect to type of infectious agent so it is recommended that the doctor should determine the dominant type of infectious agent and its susceptibility response before prescription of any antibiotic.

Keywords: Antibiotic Resistance; Antibiotic Susceptibility; Escherichia Coli; Klebsiella Specie; Urinary Tract Infection.

1. Introduction

It is a well established fact that major causative agents of Urinary Tract Infections (UTI) are bacteria; in some studies other microbes and fungal infections had also been reported (10). Escherichia coli incident was highest followed by Klebsiella pneumonia in cases of UTI, other bacterial strains reported in UTI are Staphylococcus, Enterococcus, Proteus, Pseudomonas (2). Random data analysis and studies indicate that UTI cannot be explained on the basis of age, gender or region of the world (WHO, 2017). It was noticed that women had higher incidence of UTI in most studies, nearly twenty percent women had recurrent UTI attacks (3). In males higher incidence of UTI was reported in late fifties due to different prostatic disorders, children generally suffer less UTI attacks. It was also found that fifty percent cases of UTI are hospital acquired (4). Urinary track is made up of kidneys, ureters, urinary bladder, urethra and associated glands. It is very important for osmoregulation. Any infection in UTI can damage kidneys function and could impart fetal consequences (3, 5).

Antibiotic resistance is increasing all over the world particularly in developing countries (6, 7). Pakistan being a developing country is facing the same problem. Increased resistance against cephalosporin had been reported, mainly due to over exposure to drug (8, 9). Statistics revealed that in Pakistan 18,596 standard units per 1000 people of antibiotics were used in 2014 that was doubled as compared to other neighboring countries in China it was 9894, in Bangladesh it was 9537, in Sri Lanka it was 10388 standard units per 1000 people (10). Different studies across the globe had proven that same antibiotic had different resistance or susceptibility pattern for different sites of infection. Most commonly used antibiotic for UTI are Amoxicillin + Clavulanate, Imipenam, Piperacillin+ tazobactum, Amikacin etc (11). To the best of our knowledge there is no current report regarding antibiotic resistance pattern from twin cities (Rawalpindi and Islamabad); therefore present study was designed to find the current scenario of antibiotic resistance patterns against different bacterial strains isolated from UTI patients in twin cities of Pakistan.
2. Materials and methods

The study was carried out in twin cities, in total 227 individuals suffering for UTI who visited Naveed Laboratory, Pakistan were enrolled. This study was conducted during October 2019 to June 2020 and was approved by the ethical committee of Department of Life Sciences, University of Management and Technology, Lahore, an informed written consent was taken by each participant. Midstream urine samples were taken and different biochemical test such as Gram staining, Lactose fermentation test, Motility test, Catalase test and Colony formation on MacConkey agar, Indole test, and Citrate utilization test were applied for the isolation of different microbial strains. MullarHigton agar was used for the determination of susceptibility and resistance pattern. The effect of each antibiotic had been tested on all isolates and results were compiled to determine which antibiotic was most effective against UTI.

3. Results

In present study total 227 UTI samples were tested. Among the tested samples Citrobacter spp. (1), Aerobic Gram Negative Bacteria (1), Candida spp. (11), Coagulase negative Staphylococcus (6), Enterobacter spp. (1), Enterococcus spp (2), Escherichia coli (143) and Klebsiella (63) were isolated. According to results Escherichia coli (62.7%) had highest incidence as causative agent of UTI followed by Klebsiella species (27.6%). In some patients co-infection of Escherichia coli and Klebsiella (9.2%) was also found. As both Klebsiella pneumoniae and Escherichia coli had high tendencies of antibiotic resistance; in this study their resistance and susceptibility response was evaluated against each antibiotic separately as well. According to present study Amikacin was found to be the most sensitive or susceptible antibiotic with 93.5% susceptibility followed by Imipenam with 87.4% and Piperacillin+ tazobactum with 79.8% susceptibility. Where as, Ciprofloxacin was the most resistant drug with 76.8% resistance followed by Cefixime 75.8 and Ceftriaxone 66.6% respectively (Table 1).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>TST</th>
<th>R</th>
<th>% age</th>
<th>S</th>
<th>% age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin + Clavulanate</td>
<td>196/227</td>
<td>102/196</td>
<td>52</td>
<td>94/196</td>
<td>47.9</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>198/227</td>
<td>134/198</td>
<td>67.6</td>
<td>64/198</td>
<td>32.3</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>164/227</td>
<td>126/164</td>
<td>76.8</td>
<td>38/164</td>
<td>23.1</td>
</tr>
<tr>
<td>Imipenam</td>
<td>199/227</td>
<td>25/199</td>
<td>12.5</td>
<td>174/199</td>
<td>87.4</td>
</tr>
<tr>
<td>Piperacillin+ tazobactum</td>
<td>189/227</td>
<td>38/189</td>
<td>20.1</td>
<td>151/189</td>
<td>79.8</td>
</tr>
<tr>
<td>Amikacin</td>
<td>184/227</td>
<td>12/184</td>
<td>6.5</td>
<td>172/184</td>
<td>93.5</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>194/227</td>
<td>123/194</td>
<td>63.4</td>
<td>71/194</td>
<td>36.6</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>100/227</td>
<td>64/100</td>
<td>64</td>
<td>36/100</td>
<td>36</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>113/227</td>
<td>72/113</td>
<td>63.7</td>
<td>41/113</td>
<td>36.2</td>
</tr>
<tr>
<td>Cefixime</td>
<td>149/227</td>
<td>113/149</td>
<td>75.8</td>
<td>36/149</td>
<td>24.2</td>
</tr>
</tbody>
</table>

TSR= Total Sample Tested, R= Resistance, S= Susceptibility.

More over, %age antibiotic resistance and susceptibility of each antibiotic was calculated against E.coli and K. pneumoniae separately as well. In current study total 143 E.coli samples were tested, antibiotic which were tested against minimum 100 samples was included in the analysis. Imipenam was found to be the most effect drug with 97.5% susceptibility, followed by Amikacin with 94.5% and Piperacillin+ tazobactum with 84.2% susceptibility respectively. Ciprofloxacin was most resistant drug with 81.7% resistance followed by Cefixime 74.4% and Ceftriaxone 66.1% resistance respectively (Table 2).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>TST</th>
<th>R</th>
<th>% age</th>
<th>S</th>
<th>% age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin + Clavulanate</td>
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<td>64/131</td>
<td>48.8</td>
<td>67/131</td>
<td>51.1</td>
</tr>
<tr>
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<td>45/133</td>
<td>33.8</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>104/143</td>
<td>85/104</td>
<td>81.7</td>
<td>19/104</td>
<td>18.2</td>
</tr>
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<td>13/121</td>
<td>10.7</td>
<td>118/121</td>
<td>97.5</td>
</tr>
<tr>
<td>Piperacillin+ tazobactum</td>
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<td>20/127</td>
<td>15.7</td>
<td>107/127</td>
<td>84.2</td>
</tr>
<tr>
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<td>122/143</td>
<td>8/122</td>
<td>6.5</td>
<td>115/122</td>
<td>94.5</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>131/143</td>
<td>82/131</td>
<td>62.5</td>
<td>49/131</td>
<td>37.4</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>73/143</td>
<td>44/73</td>
<td>60.2</td>
<td>29/73</td>
<td>39.7</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>86/143</td>
<td>51/86</td>
<td>59.3</td>
<td>35/86</td>
<td>40.6</td>
</tr>
<tr>
<td>Cefixime</td>
<td>98/143</td>
<td>73/98</td>
<td>74.4</td>
<td>25/98</td>
<td>25.5</td>
</tr>
</tbody>
</table>

TSR= Total Sample Tested, R= Resistance, S= Susceptibility.
The same ten antibiotics were selected for K. pneumoniae which were tested against E. coli on the basis of cut off value at minimum tested against 20 samples. Total 43 samples of K. pneumoniae were tested. It was found that Amikacin was the most susceptible drug with 94.9% susceptibility, followed by Imipenem with 85.4% and Piperacillin+ tazobactum 77.7% susceptibility against K.pneumoniae in UTI isolates. Tobramycin was the most resistant drug with 76.1% resistance followed by Cefixime 76% and Ceftriaxone with 73.6% resistance respectively (Table 3).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>TST</th>
<th>R</th>
<th>% age</th>
<th>S</th>
<th>% age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin + Clavulanate</td>
<td>56/63</td>
<td>31/56</td>
<td>53.3</td>
<td>25/56</td>
<td>44.6</td>
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<tr>
<td>Ceftriaxone</td>
<td>57/63</td>
<td>42/57</td>
<td>73.6</td>
<td>15/57</td>
<td>26.3</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>52/63</td>
<td>34/52</td>
<td>65.3</td>
<td>18/52</td>
<td>34.6</td>
</tr>
<tr>
<td>Imipenam</td>
<td>62/63</td>
<td>9/62</td>
<td>14.5</td>
<td>53/62</td>
<td>85.4</td>
</tr>
<tr>
<td>Piperacillin+ tazobactum</td>
<td>54/63</td>
<td>12/54</td>
<td>22.2</td>
<td>42/54</td>
<td>77.7</td>
</tr>
<tr>
<td>Amikacin</td>
<td>59/63</td>
<td>3/59</td>
<td>5</td>
<td>56/59</td>
<td>94.9</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>61/63</td>
<td>40/61</td>
<td>65.5</td>
<td>21/61</td>
<td>34.4</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>22/63</td>
<td>16/22</td>
<td>72.7</td>
<td>6/22</td>
<td>27.2</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>21/63</td>
<td>16/21</td>
<td>76.1</td>
<td>5/21</td>
<td>23.8</td>
</tr>
<tr>
<td>Cefixime</td>
<td>46/63</td>
<td>35/46</td>
<td>76</td>
<td>11/46</td>
<td>23.9</td>
</tr>
</tbody>
</table>

TSR= Total Sample Tested, R= Resistance, S= Susceptibility.

The comparison of over all %age antibiotic resistance and individual %age of E. coli and K. pneumoniae clearly indicated that drug resistance against different antibiotics varies with the type of pathogenic bacteria (Fig 1).

4. Discussion

In present study antibiotic resistance and susceptibility patterns of Klebsiella and E. coli against top ten antibiotics was calculated. The study results highlighted that in a developing country like Pakistan it is very important to evaluate the efficacy of prescribed antibiotic. This study is a small contribution to evaluate change in resistance and susceptibility patterns with the type of disease causing bacterial stains, as very limited data in this regard is available from Pakistan.

In present study E. coli and Klebsiella were the main causative agents for UTI and their incidence was 62.7% and 27.6%. In an other study from Pakistan the incident of E. coli was reported as 40% in 2015 (11). In a study carried out at tertiary care hospital of Pakistan the incidence of E. coli was reported as high as 80% in UTI patients (12). In Iran it was reported as 64% in 2007 and in next year it was reported as 60.9% (13). In USA overall incidence of E.coli in UTI was found from 75-90% (14).

In a study from Liaquat University Hospital Jamshoro reported incident of Klebsiella 39.1% in UTI patients (9). In India, Nepal, Ethiopia and Nigeria the incidence of Klebsiella was 14.4%, 15.7%, 31.5% and 3.5 % respectively in cases of UTI (15-18).

For the treatment of E. coli and Klebsiella different antibiotics are prescribed in Pakistan and all over the world. In this study Ceftriaxone, Ciprofloxacin, Imipenam, Amikacin, Ceftazidime, Levofloxacin, Tobramycin, Cefixime and combination of antibiotics like Amoxicillin + Clavulanate and Piperacillin+ tazobactum were used. Studies indicated that Imipenem was most widely used antibiotic in countries such as Iran, India, Estonia, Lebanon, Nigeria, Jordan Turkey, Saudi Arabia and Pakistan against bacterial infections including UTI (11,19-29).

It was found in present study that over all and against E.coli and Klebsiella, Imipenem has susceptibility 87.4%, 97.5% and 85.4% respectively in UTI infection. In other studies across the globe sensitivity was 100 % in Nigeria, 90 % in Estonia, India and Iran while 70% in Jorden (11,19-20, 22-25, 27-29). Different studies from Pakistan, India and Turkey showed variable results but in all studies susceptibility of Imipenem was reported high; it was reported between 95% to 98% in these regions which is comparable to our results (9, 26, 29-31).

In present study Ciprofloxin resistance was 81.7%, 76.8% and 65.3 against all isolates, E. coli and Klebsiella respectively. Other similar studies on UTI isolates carried out in Uganda (87%), Ethiopia (80%), Nepal (49.4%) and Jorden (75%) which showed higher sensitivity than present study (11, 15-17, 23-24, 27, 29, 32-33).

In present study against Amikacin over all susceptibility was 93.5%, against E. coli % it was 94.5% and against Klebsiella sensitivity was reported as 85.4%. Our findings were similar to other susceptibility reports from India (74%), Estonia with 98%, Nepal with 87 %, Nigeria, Iran with 100% (17, 23-24, 33-37). For E.coli susceptibility was also high and it was reported over 90% in most studies (9, 26, 29-31).
Tobramycin was resistant in 63.7% isolates in present study. These results were quite contrary to other finding in which %age susceptibility was much higher approximately 70% in Pakistan, India and Iran (13, 26, 38).

Resistance against Ceftriaxone was 67.6%, whereas % susceptibility ranges from 30-60% in different regions (13, 18, 26-27, 32, 36, 39). In present study against E.coli resistance was 66.1%.

In current study over all UTI causing isolates showed 63.4% resistance against Ceftrizidine, for Klebsiella resistance was 65.5% and for E. coli it was 62.5%. Its resistance was much higher similar to other studies reported from Nigeria, Iran, India and Lebanon where it was as high as 80% (8, 15-16, 18, 20, 24, 35).

It was found in this study that Cefixime and Levofloxacin were 75.8% and 65% resistant respectively. These results were similar to other studies in the region. Similar patterns were reported from other regions like Nepal, India, Iran and Nigeria for E. coli and Klebsiella both strains (11, 15-16, 24, 31, 35).

It was found that against Amoxicillin + Clavulanate 52% samples showed resistance. In our study against E.coli % resistance was 48.8% and against Klebsiella it was 53.3%. Other studies from Pakistan and Saudi Arabia showed higher resistance than reported in present study (11, 30).

Our results showed that Piperacillin and Tazobactum were 78.9% susceptible against common pathogens. Therefore, it was found in this study that Cefixime and Levofloxacin were 75.8% susceptible against common pathogens.

Results showed that same antibiotics had different susceptibility response for different pathogens in UTI isolates. Therefore, it is recommended that this study should be extended on national level and patients should be screened not only for UTI but all other type of infection as well. Pakistan is a poor country with low literacy rate and poor awareness about contact hygiene so it is very important that increasing drug resistance response of gram negative disease causing agents should be controlled.

5. Conclusion

Pakistan ranks highest in antibiotic consumption per 1000 people in South Asia, such large scale antibiotic consumption makes Pakistan vulnerable for developing multi drug resistance (MDR). Since no study was reported on MDR pattern in isolates from UTI in last five years from Islamabad, this study was an effort to highlight the need of pre-screening for the type of pathogen before prescribing any drug. Results showed that some antibiotics had different susceptibility response for different pathogens in UTI isolates. Therefore, it is very important to prescribed targeted drug in case of UTI infection. In 2017 WHO announced antibiotic resistance among top ten emerging health issues. It is recommended that this study should be extended on national level and patients should be screened not only for UTI but all other type of infection as well. Pakistan is a poor country with low literacy rate and poor awareness about contact hygiene so it is very important that increasing drug resistance response of gram negative disease causing agents should be controlled.

Declarations section

Ethics approval and consent to participate
The study protocols and informed consent documents were approved by the Institutional Bioethics Review Committee (IBRC).

Consent to publish
Informed written consent for publication was obtained from each participant.

Availability of data and materials
The datasets used and/or analyzed during the study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

References


