Research Article

Antimicrobial Resistance Pattern against *Staphylococcus aureus* in Environmental Effluents

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INTRODUCTION

*Staphylococcus aureus* is one of the ubiquitous microorganisms and are frequently associated with various infections. Antibiotic resistant *Staphylococcus aureus* isolates pose an unbelievable challenge to both veterinary and human health professionals because they have a pessimistic therapeutic impact (Brouillette and Malouin, 2005). The indiscriminate usage of antibiotics correlates with the emergence, maintenance and transmission of antibiotic resistant traits within pathogenic strains (Shitandi and Sternees, 2004). These resistance traits are coded by particular genes that may be carried on the bacterial chromosome, plasmids, on gene cassettes that are incorporated into integrons (Rychlik et al., 2006), thus are transferred among isolates an easy manner. National estimates from 2001 to 2002 in USA suggest that 32.4% of individuals are colonized with *Staphylococcus aureus*, and 0.8% of individuals were colonized with MRSA (Graham et al., 2006). Biomedical wastes pose great danger in hospital as estimated that 20% of the biomedical waste is “highly infectious” and is a hazard since it is often disposed of into the sewage system, drains or water bodies. Such poor sanitation has serious consequences for the health of the residents and a report suggests that ”most of the child mortality” could be related with this problem (Bhuiya, 2007). Resistance bacterial contamination of waste waters worldwide is estimated to cause millions of gastrointestinal and acute respiratory infections (Shuval, 2003) and numerous skin infections (Yau et al., 2009) every year. Unlike typical hospital associated strains, some strains of community associated *Staphylococcus aureus* can cause infections in healthy people with no traditional risk factors for infection (Gorwitz, 2008). Even though most patients are treated as outpatients, hospitalization rates remain substantial (Jarvis et al., 2007). Similarly, abattoirs are generally known all over the world to pollute the environment either directly or indirectly from their various processes (Adelekan, 2002). Health risks of the effluents of slaughterhouses play role in environment degradation and to dissemination of pathogenic resistance microorganisms during the rainy and dry seasons which not manage properly. Such a practice has led to misuse of antimicrobial drugs with the associated high prevalence of drug resistance among the staphylococci (Okuma, 2002). Multiple antibiotic resistant *Staphylococcus aureus* strains have been isolated from milk, meat, water, human and environmental samples in many parts of the world (Pesavento et al., 2007) but minimal concern gather towards multidrug resistance *Staphylococcus aureus* isolates from effluents in Indian subcontinent. A majority of the prescribers in Bangladesh particularly in veterinary practice made diagnosis of infection based on clinical assessment and suspect a microbial etiology due to inadequate diagnostic facilities and frequently choose higher antibiotics (Faiz and Rahman, 2004). The important factors associated with resistant bacteria with poor hospital hygiene, overcrowding, lack of resources for infection control.

and lack of personnel trained in controlling infection in both medical and veterinary hospital (Faizer and Bashor, 2011).

Bangladesh is the populous country having its place at ninth most densely populated countries in the world. In particular, from 2010–2015 the projected urban population growth rate is 3% (UNdata, 2012). With this population growth, there is an increasing problem of waste management particularly in the larger cities due to inadequate budget and awareness. The minimal waste collection coverage forces majority of the waste to be dumped in open lands or drainage system. These wastes are not disposed properly, where general wastes are often mixed with hazardous waste such as hospital waste, slaughterhouse waste and other environmental wastes. Frequently these wastages clog together due to solid waste and submerge the public place and cultivable land and contaminated the surroundings with resistance microbes. Therefore the current studies were taken out to isolates the Staphylococcus aureus and find out their antibiotic resistance pattern from environmental effluents.

MATERIALS AND METHODS

Study Area

The study was carried out in Chittagong Metropolitan area had an estimated population of more than 6.5 million people. At present government, non-government hospitals and clinics have provided to the medical service for the city civilian and also a number of veterinary hospitals are present for animal health care. On the other hand, some slaughterhouses are also established scattered within the city. Six (6) medical hospitals, five (5) veterinary hospitals and five (5) slaughterhouses were selected randomly from the list of Chittagong City Corporation.

Study Duration and Sample Collection

The study was conducted during the period of September to December, 2012. Samples were collected from final effluents of medical hospitals, veterinary hospitals and slaughterhouses and 230ml pre-sterilized glass bottles were used to transport the samples to PRTC (Poultry Research and Training Center) laboratory for analysis.

The collected samples were preserved in a refrigerator chiller at 4°C during the research period at PRTC laboratory.

Media Used

Nutrient broth (Oxoid Ltd., pH 7.4±0.2) was used as primary enrichment media for Staphylococcus. Selective media Mannitol salt agar (Merck, pH 7.4±0.2) was used for isolation of the bacteria and Muller Hinton agar (Biotec, pH 7.3±0.1) used for determination of antibiogram.

Isolation and Identification of Organism

For the isolation of Staphylococcus aureus, 1ml of water sample was inoculated in screw cap test tube containing nutrient broth (primary enrichment media) and incubated overnight at 37°C. After primary enrichment sample from nutrient agar was streaked on mannitol salt agar and incubated for 24 hours at 37°C. After overnight incubation the bacterial growth was observed and the positive samples colony were selected based on colony morphology and confirmed by Gram’s staining.

Gram Staining

Gram’s staining was performed as per procedures described by Merchant and Packer (1969) to determine the size, shape and arrangement of bacteria.

Catalase Test

Pure culture of each isolate was picked using a sterile loop from the agar slant and mixed with a drop of 3% H2O2 on a clean glass slide. Bubbles of oxygen were liberated within a few seconds.

Coagulase Test

Slide Coagulase Test

Dense suspensions of Staphylococci from culture were made on two ends of clean glass slide. One was labeled as “test” and the other as “control”. The control suspension serves to rule out false positivity due to auto agglutination. The test suspension was treated with a drop of EDTA treated horse plasma and mixed well. Agglutination or clumping of cocci within 5–10 seconds was taken as positive. Some strains of Staphylococcus aureus may not produce bound coagulase, and such strains must be identified by tube coagulase test.

Tube Coagulase Test

The tube coagulate test was performed in sterile tubes by adding 0.5ml of isolates of Staphylococcus grown on tryptone soya broth (TSB) at 37°C for 24h to 0.5ml of Goat plasma. After mixing by gentle rotation, the tubes were incubated at 37°C along with a negative control tube containing was evaluated at 30 minutes intervals for the first 4h of the test and then after 24h incubation. The reaction was considered was positive if any degree of clotting from a loose clot to a solid clot that is immovable when the tube is inverted (tilted) was visible within the tube and no degree of clotting would be taken as negative.

CS test at Muller Hinton Agar

After confirmation of isolates as Staphylococcus aureus, antimicrobial susceptibility of the isolates were determined by using the micro disc diffusion method, and the method was used according to guidelines established by Clinical and Laboratory Standards Institute (CLSI) (2010).

Data Analysis

Data obtained was imported to the Microsoft Office Excel–2007 and transferred to the software STATA/IC–11 for analysis. Descriptive statistics was done and expressed as percentages of resistance pattern of antimicrobials.

RESULTS

The results of isolation and identification of Staphylococcus aureus in culture of Mannitol salt agar, Gram staining, Catalasate and Coagulase test were given in table–1. The Staphylococcus aureus ferments mannitol and turned the medium yellow. The positives isolated from mannitol salt agar microscopically detected as Gram–positive, cocccid as clustered of grapes. The colonies were characterized as round, smooth and glistening that was positive in 5 Medical hospital, 2 Veterinary hospital and 4 slaughterhouse samples, as mannitol salt agar is selective media for Staphylococcus spp. Catalase and Coagulase test was done for conformation of Staphylococcus aureus and find all the sample positive.

Table–2 represents the results of CS-test for isolates of Staphylococcus aureus. Ten antibiotics were used from which AMX, CH, CL, CN, TA and PF were resistance to all five medical hospitals isolates. ENR, GEN and K were intermediate sensitive to I medical hospital isolates but shown resistance to rest of the three. GEN, K and N were sensitive to medical hospital isolates. AMX, CH, CL, CN, TA and PF were resistance to all Veterinary hospital isolates, K was intermediate sensitive and ENR, GEN and N were sensitive to one isolates. All Staphylococcus aureus isolates from slaughterhouses were able to
shown resistance against AMX, CH, CL, TA, ENR and PF. Sensitivity to CN was only found in one sample and was resistance to others.

The prevalence of antibiotic resistance pattern against *Staphylococcus aureus* positive isolates were shown in Table 3. The prevalence of resistance among antibiotics were 100% to AMX, CH, CL, CN, PF and TA followed by ENR (80%), GEN (40%), K (40%) and N (40%) in the isolates of medical hospital. The prevalence of antimicrobial resistance pattern against *Staphylococcus aureus* were 100% to AMX, CH, CL, CN, TA and PF followed by ENR (50%), GN (50%), K (50%), N (50%) in the isolates of veterinary hospital. The level of resistance for slaughterhouse *Staphylococcus aureus* isolates to specific antibiotics were 100% to AMX, CH, CN, TA and PF followed by 75% resistance in CL, ENR and K, 50% to Gentamicin and 25% to N.

**DISCUSSION**

*Staphylococcus aureus*, a spherical, aerobic, Gram positive, catalase positive, is an opportunistic pathogen for both human and livestock population, and is one of the most frequent etiological sources of hospital and community infections. Generally, *Staphylococcus aureus* is responsible for superficial pyogenic infections as abscess, systemic infections such as endocarditis, osteomyelitis and pneumonia, and toxicosis such as food poisoning or toxic shock syndrome after ingestion of contaminated food. The isolates of *Staphylococcus aureus* from medical hospitals, veterinary hospitals and slaughterhouses were tested to find out the antimicrobial resistance pattern. The effluents were contaminated by the large number of patients in both human and animal hospitals, and different species of animals in slaughterhouses, and high possibilities of contamination of existent *Staphylococcus aureus* in the effluents of those places. Antimicrobial resistance pattern of the *Staphylococcus aureus* from samples of hospitals and slaughterhouses showed high resistance levels. The level of resistance is higher than the findings described by Virdis et al. (2010) and that might be due to variation of environment and contaminated effluents. A study reported that, *Staphylococcus* resistance to oxacillin, penicillin and ampicillin was 100% and cephalothin was 92.4% (Bukhari et al., 2011) and those were agreed to present findings. Kanamycin resistance was not acute in our present research and effectiveness was still showed against hospital isolates but minor sensitivity was showed against slaughterhouse isolates. Our results support the findings of the prevalence of kanamycin resistance bacteria in drinking water from a residential area and closed to the values.

**Table 1: Isolation and Identification of *Staphylococcus aureus* in culture on Mannitol salt agar, Gram staining, Catalase and Coagulase test**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mannitol Salt Agar</th>
<th>Microscopic features</th>
<th>Catalase test</th>
<th>Coagulase test</th>
</tr>
</thead>
<tbody>
<tr>
<td>MH N-6</td>
<td>5 (S)</td>
<td>(1) ND</td>
<td>5 (S)</td>
<td>5 (S)</td>
</tr>
<tr>
<td>VH N-5</td>
<td>2 (S)</td>
<td>(3) ND</td>
<td>2 (S)</td>
<td>2 (S)</td>
</tr>
<tr>
<td>SH N-5</td>
<td>4 (S)</td>
<td>(3) ND</td>
<td>4 (S)</td>
<td>4 (S)</td>
</tr>
</tbody>
</table>

MH: Medical Hospital; VH: Veterinary Hospital; SH: Slaughterhouse; S: Positive; –: Negative; ND: Not detected

**Table 2: CS-test for isolates of *Staphylococcus aureus***

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Medical Hospital</th>
<th>Veterinary Hospital</th>
<th>Slaughterhouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMX</td>
<td>5 (100%)</td>
<td>2 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>CH</td>
<td>5 (100%)</td>
<td>2 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>CL</td>
<td>5 (100%)</td>
<td>2 (100%)</td>
<td>3 (75%)</td>
</tr>
<tr>
<td>CN</td>
<td>5 (100%)</td>
<td>2 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>ENR</td>
<td>4 (80%)</td>
<td>1 (50%)</td>
<td>3 (75%)</td>
</tr>
<tr>
<td>GEN</td>
<td>2 (40%)</td>
<td>1 (50%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>K</td>
<td>2 (40%)</td>
<td>1 (50%)</td>
<td>3 (75%)</td>
</tr>
<tr>
<td>N</td>
<td>2 (40%)</td>
<td>1 (50%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>TA</td>
<td>5 (100%)</td>
<td>2 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>PF</td>
<td>5 (100%)</td>
<td>2 (100%)</td>
<td>4 (100%)</td>
</tr>
</tbody>
</table>

AMX: Amoxicillin; CH: Cefradin, CL: Colistin; CN: Ceftalexin; ENR: Enrofloxacin; GEN: Gentamicin; K: Kanamycin; N: Neomycin; TA: Oxetetracycline; PF: Pefloxacin

**Table 3: Prevalence of antibiotic resistance pattern against *Staphylococcus aureus* positive isolates**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Medical Hospital</th>
<th>Veterinary Hospital</th>
<th>Slaughterhouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMX</td>
<td>5 (100%)</td>
<td>2 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>CH</td>
<td>5 (100%)</td>
<td>2 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>CL</td>
<td>5 (100%)</td>
<td>2 (100%)</td>
<td>3 (75%)</td>
</tr>
<tr>
<td>CN</td>
<td>5 (100%)</td>
<td>2 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>ENR</td>
<td>4 (80%)</td>
<td>1 (50%)</td>
<td>3 (75%)</td>
</tr>
<tr>
<td>GEN</td>
<td>2 (40%)</td>
<td>1 (50%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>K</td>
<td>2 (40%)</td>
<td>1 (50%)</td>
<td>3 (75%)</td>
</tr>
<tr>
<td>N</td>
<td>2 (40%)</td>
<td>1 (50%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>TA</td>
<td>5 (100%)</td>
<td>2 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>PF</td>
<td>5 (100%)</td>
<td>2 (100%)</td>
<td>4 (100%)</td>
</tr>
</tbody>
</table>

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of resistance for isolates from college drinking water but differ from the values obtained from hospital drinking water (Samra et al., 2009). The variation in prevalence of antimicrobial resistance patterns might be due to differences in hygienic condition and contamination of water in study area.

Chu et al. (2012) reported 137 staphylococcal strains were identified predominantly from milk, and in the vagina, anus, and nasal cavity. Nearly all isolates were found susceptible to enrofloxacin, gentamicin, neomycin, and vancomycin and in addition, over 60% of the isolates were also resistant to pefloxacin, tetracycline, oxytetracycline, and the significance of culture and the importance of resistant bacteria resulting from the use of antibiotics in the various fields has to be measured. For this purpose, it is important to make a more detailed assessment of the significance of culture-dependent and laboratory-based methods in relation to conditions found in the environment. Based on the above resistance pattern of antimicrobial agents on environmental samples, measures should be taken to avoid or control the antimicrobial resistance and improve the public health.

ACKNOWLEDGEMENTS
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COMPETING INTERESTS
Authors declare that they have no competing interests.

REFERENCES
UN data (2012). Country Profile: Bangladesh.