Prevalence and Antimicrobial Susceptibility of \textit{Staphylococcus aureus} Isolated from Retail Chicken Meat in Chennai, India

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ABSTRACT

The present study was conducted to evaluate the prevalence of \textit{S. aureus} in retail chicken meat marketed in Chennai, Tamil Nadu and to determine the antimicrobial susceptibility pattern of these isolates. A total of 120 meat samples were collected from different retail outlets and it was observed that 66.67 (80/120) per cent of the samples were positive for the presence of \textit{S. aureus} based on biochemical characterization. The \textit{S. aureus} count in chicken meat samples (160) ranged from $2.8 \times 10^2$ to $1.5 \times 10^4$ CFU/ g with an overall average of $2.98 \times 10^3$ CFU/ g. Antimicrobial susceptibility testing of these isolates against 16 antimicrobials revealed that all the isolates were resistant to Ampicillin (100%) followed by Tetracycline (87.50%), Amoxicillin (77.50%), Erythromycin (72.50%), Polymyxin- B (70%), Cefoxitin (67.50%), Novobiocin (67.50%), Oxacillin (65%) and Ciprofloxacin (50%). Majority of the chicken isolates were sensitive to Gentamicin (92.50%), followed by Vancomycin (87.50%), Chloramphenicol (82.50%), Neomycin (80%), Kanamycin (77.50%), Co-trimoxazole (62.50%) and Methicillin (55%). Results of Multiple Antibiotic Resistance (MAR) Index revealed that 99.38 per cent of the isolates (119/120) had MAR index of above 0.2 and only one isolate had index below 0.2. The results clearly indicated higher prevalence of multidrug resistant \textit{S. aureus} in retail Chicken meat from public health point and the level of hygiene prevalent in these outlets.

Keywords: \textit{S. aureus}, Chicken meat, Antimicrobial susceptibility, MAR index

In the present day context meat and meat products plays a vital role in providing food security as well as balanced nutrition at an affordable price to people in developing countries. However, because of its high nutritive value, meat is highly susceptible to microbial growth resulting in faster spoilage. The muscle of the animal is sterile before slaughter and generally gets contamination during the slaughter process viz., hide, contaminated work surfaces, butchers, quality of water and environment (Lues \textit{et al.}, 2007; Jones \textit{et al.}, 2008). Furthermore, in developing countries like India, where meat is being sold in open markets contamination of meat is inevitable since they are exposed to various microorganism and hence proper hygiene should be in place to minimize the level of contamination. Among the various pathogens that enter meat through contamination, \textit{S. aureus} is the most prevalent since it is a natural flora in skin and nasal cavity of human and animals (Pal, 2001).

The prevalence of \textit{S. aureus} in retail meat and meat products have been documented by several workers and data suggests that the rate of prevalence is much higher in developing countries compared to that of developed countries indicating the lack of hygiene during slaughter and further processing (Ruban \textit{et al.}, 2012; Sharma and Chattopadhay, 2015; Gayathri and Anu, 2015). In the recent decades, antimicrobial resistance of bacteria has
been considered as a major public health problem due to indiscriminate use of antibiotics in poultry production as growth promoters, resulting in development of antibiotic resistant strains of *S. aureus*. Hence, the present study was designed to evaluate the prevalence of *S. aureus* in retail chicken meat marketed in Chennai, Tamil Nadu, India and to study the antibiotic susceptibility pattern of these isolates against commonly used antibiotics in both human and animals.

**MATERIALS AND METHODS**

The protocol and methodology used in the present study for isolation and characterization of *S. aureus* from Chicken meat was carried out with approval from Institutional Biosafety Committee of Tamil Nadu Animal and Veterinary Sciences University, Chennai.

**Study area and Source of Material**

A total of 120 chicken meat samples collected in sterile containers from different retail outlets in Chennai city (South, Central and North Zone) were used in his study.

**Isolation of *Staphylococcus aureus***

Isolation of *Staphylococcus aureus* was done as per the standard procedure (ISO standard 6888/1:1999; 6888/2: 1999). In brief, ten grams of each sample was added to 90 ml of sterile Brain Heat Infusion broth supplemented with 10% NaCl and enriched for 8-10 hours at 37°C. The enriched samples were streaked onto Baird Parker agar base (Himedia, India) and suspected colonies were identified by Gram staining, catalase test, Mannitol fermentation, Coagulase and thermonuclease test as per standard protocol. The *S. aureus* count of the meat samples were determined by procedure outlined by Bhandare *et al.* (2007).

**Antimicrobial susceptibility test**

Antimicrobial susceptibility test was done as per the standard disc diffusion method of Bauer *et al.* (1966). All the isolates confirmed by biochemical test were tested for their susceptibility pattern against 16 antimicrobial discs (Himedia, India) in Muller Hinton agar plates. The interpretation of zone diameter was carried out according to Clinical Laboratory Standard Institute (CLSI) manual (2010).

**Multiple Antibiotic Resistance Index (MAR)**

The MAR index was calculated as per the procedure outlined by Krumperman (1983) by the formula a/b, where ‘a’ represents the number of antibiotics to which the particular isolate is resistant and ‘b’ the number of antibiotics to which the isolate is exposed.

**RESULTS AND DISCUSSION**

**Prevalence of *S. aureus***

The results of the study revealed that out of the 120 chicken meat samples screened 102 (85%) of the samples were contaminated with *Staphylococci* sp., of which 92 (76.67%) isolates were Coagulase positive *Staphylococci* (CoPS) based on coagulase test and based on Mannitol fermentation and thermonuclease test its observed that 80 samples were positive for presence of *S. aureus* with a prevalence rate of 66.67 per cent. The results of our study were in accordance with the findings of Kitai *et al.* (2005) in Japan (65.8%), Adesiji *et al.* (2011) in Nigeria (80%), Ruban *et al.* (2012) in Bangalore, India (82-100%), Islam *et al.* (2014) in Bangladesh (88-95%), Gencay *et al.* (2010) in Turkey (55%) and Ramya *et al.* (2015) in Hyderabad (53%). The above researchers have attributed the high prevalence of *S. aureus* to poor personal hygiene, quality of water used for processing and improper or no sanitation of the knives as well as working surfaces in the retail outlets. Hence, the high prevalence in the present study clearly indicates poor hygiene practiced during the slaughter and processing of Chicken in the retail outlets of Chennai.

However, Contrary to the findings of our study, lower prevalence have been reported by Gayathri and Anu (2015) in Chennai (10%), Arul Kumar and Saravanan (2011) in Namakkal (6.67%), Hanson *et al.* (2011) in Iowa (17.8%), Bhargava *et al.* (2011) in USA (25%) and Wang *et al.* (2013) in China (24.2%). The variation in the prevalence might be due to number of samples screened, sampling procedure as well as hygienic condition of retail outlets in different countries.
Antimicrobial Susceptibility

The antibiotic susceptibility pattern of *S. aureus* isolated from Chicken meat from different retail outlets of Chennai city are presented in Table 1.

**Table 1:** Antibiotic susceptibility pattern of *S. aureus* isolated from chicken in retail outlets of Chennai city

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Chicken isolates (n=80)</th>
<th>Sensitive</th>
<th>IM</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methicillin</td>
<td>44 (55.00)</td>
<td>10 (12.50)</td>
<td>26 (32.50)</td>
<td></td>
</tr>
<tr>
<td>Oxacillin</td>
<td>28 (35.00)</td>
<td>—</td>
<td>52 (65.00)</td>
<td></td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>26 (32.50)</td>
<td>—</td>
<td>54 (67.50)</td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>70 (87.50)</td>
<td>—</td>
<td>10 (12.50)</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>74 (92.50)</td>
<td>—</td>
<td>6 (7.50)</td>
<td></td>
</tr>
<tr>
<td>Neomycin</td>
<td>64 (80.00)</td>
<td>—</td>
<td>16 (20.00)</td>
<td></td>
</tr>
<tr>
<td>Kanamycin</td>
<td>62 (77.50)</td>
<td>—</td>
<td>18 (22.50)</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>66 (82.50)</td>
<td>2 (2.50)</td>
<td>12 (15.00)</td>
<td></td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>50 (62.50)</td>
<td>8 (10.00)</td>
<td>22 (27.50)</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>22 (27.50)</td>
<td>18 (22.50)</td>
<td>40 (50.00)</td>
<td></td>
</tr>
<tr>
<td>Polymyxin- B</td>
<td>24 (30.00)</td>
<td>—</td>
<td>56 (70.00)</td>
<td></td>
</tr>
<tr>
<td>Novobiocin</td>
<td>26 (32.50)</td>
<td>24 (30.00)</td>
<td>30 (67.50)</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>22 (27.50)</td>
<td>22 (27.50)</td>
<td>36 (72.50)</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>—</td>
<td>—</td>
<td>80 (100.00)</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>18 (22.50)</td>
<td>—</td>
<td>62 (77.50)</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>10 (12.50)</td>
<td>—</td>
<td>70 (87.50)</td>
<td></td>
</tr>
</tbody>
</table>

IM- Intermediate, Values within parenthesis indicates percentage.

In the present study it was observed that of 80 *S. aureus* isolates from chicken tested all the isolates were resistant to Ampicillin (100%) followed by Tetracycline (87.50%), Amoxicillin (77.50%), Erythromycin (72.50%), Polymyxin- B (70%), Cefoxitin (67.50%), Novobiocin (67.50%), Oxacillin (65%) and Ciprofloxacin (50%). Majority of the chicken isolates were sensitive to Gentamicin (92.50%), followed by Vancomycin (87.50%), Chloramphenicol (82.50%), Neomycin (80%), Kanamycin (77.50%), Co-trimoxazole (62.50%) and Methicillin (55%). Intermediate resistance was observed in some isolates towards Novobiocin, Erythromycin, Ciprofloxacin and Methicillin. The results were in agreement with the findings of Daka et al. (2012), Datta et al. (2012), Montaz et al. (2013), Islam et al. (2014); Modak et al. (2014) who have also reported that majority of the *S. aureus* isolates from meat samples from retail outlets were completely resistant to Ampicillin, Tetracycline, Amoxicillin, Penicillin, Erythromycin and Novobiocin.

The higher resistance of isolates to Ampicillin, tetracycline and erythromycin in the present study may be attributed to the fact that these are the commonly used antibiotics for treatment of skin and nasal infections in both animals as well as human beings. However, higher sensitivity to Gentamicin and Chloramphenicol might be due to the fact that these antibiotics are seldom used in the present day context. The Variations in sensitivity pattern of the isolates may be attributed to the use of different antibiotics in various countries for prophylaxis as well as growth promotion.

**Multiple Antibiotic Resistance Index (MAR)**

MAR index provides an indication about resistance of an organism to multiple antibiotic and value higher than 0.2 are considered to have originated from high risk sources and has high risk potential in consuming such foods and values less than 0.2 indicated that the strains originated from sources where antibiotics are seldom used and has lower risk potential (Krumperman, 1983). In the present study it was observed that of the 80 isolates, 2 isolates had MAR index of 0.1, 6 isolates (7.50%) had 0.3, 12 isolates (15.0%) had 0.4, 20 isolates (25.0%) had 0.5, 26 isolates (32.5%) had 0.6, 10 isolates (12.5%) had 0.7 and 4 isolates (5%) had MAR index of 0.8. Higher MAR index in chicken might be attributed to indiscriminate use of antibiotics as growth promoter because of paradigm shift in poultry production from backyard towards industrialized production. Similar findings have been reported by Islam et al. (2014), Abdulrahman et al. (2015); Mashouf et al. (2015) who have observed that majority of the *S. aureus* isolated from chicken are multidrug resistant and have higher MAR index.

**CONCLUSION**

The result of the present study indicates the poor hygienic condition prevalent in the retail outlets in Chennai city catering to the needs of the consumers. In addition, these isolates are multidrug resistant to commonly use antibiotics in both human as well as animals. Hence, strict measures need to be put in place to reduce contamination as well as control use of antibiotics in poultry production to prevent emergence of antibiotic resistant pathogens in meat.
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