Detection of Drug-resistant *S. aureus* from Poultry Samples Collected from Different Areas of Bangladesh

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Authors' contributions

Article Information

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ABSTRACT

Staphylococcus aureus is gram-positive cocci that can cause foodborne illness which can be transmitted by chicken meat, both raw and undercooked; consumption of which may cause infection and/or toxicity in consumers. This current study was conducted for the detection of the prevalence of *S. aureus* in three types of poultry samples which included chicken meat, chicken eggs and droppings. Samples were aseptically collected from different rural and urban areas of 8 districts of Bangladesh in triplicate collection method which was conducted in the Centre for Excellence, Department of Microbiology, Primeasia University, Dhaka, Bangladesh to detect the Multi Drug-

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Resistant (MDR) *S. aureus*. Isolation was done by using 7.5% Sodium Chloride broth for enrichment and Mannitol salt agar after enriching samples in Buffer Peptone water. Antibiogram was done by using Kirby Bauer Method on Muller Hinton agar. The current study revealed 68% (17/25), 84% (21/25) and 52% (13/25) of *S. aureus* in chicken meat, eggs and dropping, respectively. Around 75.47% isolates (40/53) showed 100% similarity with *S. aureus* from the generated Dendrogram based on biochemical data. Cefixime (62.67%) showed the highest sensitivity against all of the isolates while Penicillin (86.62%) exhibited the highest resistance. Moreover, 59.62% of isolates were resistant to 5 or more drugs (Multidrug-Resistant). Poultry products such as meat and eggs were contaminated with drug-resistant *S. aureus* which can cause serious health effect to the consumer. The drug-resistant pathogen in droppings helps to indicate the spread of drug-resistant isolates in the environment. Therefore, proper sanitation measures should be taken to ensure the quality of the products during slaughtering, collecting eggs or disposing of poultry litter.

1. INTRODUCTION

The poultry industry is a dynamic sector for providing a source of high protein-containing food among people worldwide. Poultry refers to all domesticated bird in agriculture which provide egg-laying and meat products including chicken, duck, turkey, pigeons, quail, etc. Contributing to 38% of world meat consumption, poultry is in the second position for consumed meat around the world [1,2]. For the widespread human consumption of poultry meats and eggs, the control of microbial contamination is necessary [3]. Poor hygiene, sanitation and poor to nonexistent microbiological monitoring are major contributing factors for various type of food born infection as well as promoting the growth of harmful drug-resistant pathogens in them [4,5]. Food poisoning and intoxications caused by microorganisms each year cause severe repercussion to public health along with economic losses of related industries and personnel. In a report in 2015, WHO stated that each year out of 600 million infected patients approximately 420,000 people die due to pathogens whose origin are foodborne, mostly caused by *Salmonella* sp., *Campylobacter* sp., *Listeria* sp., *Vibrio cholera*, and *Staphylococcus aureus* [6]. Majority of these organisms are available in poultry samples of various origins [7]. Several pathogenic diseases such as dermatitis, pneumonia, septicemia, osteomyelitis, and meningitis can cause infections both in humans and swine. Also, bovine mastitis in cattle and bumblefoot disease in poultry can be caused by enterotoxigenic strains of coagulase-positive staphylococci, mainly *S. aureus* [8,9]. Staphylococcal disease association varies with the source of infection (farms and other places) or infection in different body parts such as skin, muscles and yolk sac. Immunocompromised patients are often more prone to staphylococcal infections [10]. In Bangladesh, poultry farming and business are quite popular because of its low investment, more profit and easy farming methods. However, in most of the cases, it is managed by unprofessional and uneducated people. The lack of microbiological knowledge and improper use of antibiotics in poultry feed as a growth promoter in Bangladeshis are giving rise to several complications. In last few decades' unregulated uses of antibiotics as a preservative, probiotic and growth promoter are contributing to the rise of drug-resistant pathogens in the poultry industry and have turned to permit the spread of these drug-resistant strains into public health, surrounding fauna and environment [11,12]. Though rich in nutritional value, chicken meat is now quite often contaminated with drug-resistant pathogens and also contains a trace of antibiotics that can be used as growth promoters in poultry farms [13]. The previous report showed the prevalence of enterotoxigenic zoonotic *S. aureus* distributed into food from poultry products, farmworkers, communities, hospitals and industries in various countries [14]. The outbreak of *S. aureus* among 2% food handlers in Italy, 12% of flight catering members in Finland, 19% in Chile among restaurant worker and more than 61% of fish processing factory staffs in India were reported [15-18]. Between 2002 and 2003, retail data observed in Japan indicated 17.6% of raw chicken meat infested with enterotoxigenic *S. aureus*. It can indicate to the staphylococcal outbreak in future which might result in increased mortality and morbidity [19]. In Bangladesh, *S. aureus* and MRSA isolated from ready to eat foods, Milk, raw chicken meat, fish and eggs showed a significant increase in the

last few years. *S. aureus* strains isolated from poultry origin exhibited high resistance to penicillin, Chloramphenicol, Erythromycin and other antibiotics, creating new concerns for public health [20-22].

Based on data and depicting the situation above this study aimed to isolate drug-resistant *S. aureus* from chicken meat, egg and dropping on litter obtained from the farm, slaughterhouses situated in different rural and urban areas of Bangladesh.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 75 samples were collected from farms which are suppliers for the slaughter house and small retailers, who supply them to the local community, from November 2018 to April 2019. 25 meat, 25 egg, and 25 dropping samples were collected in triplicate and analyzed for detection of drug-resistant *S. aureus*. Samples were collected from different locations around Bangladesh based on the following criteria such as most popular customer shop, wholesales farms and market areas in selected areas (Table 1). All meat samples were slaughtered by the butcher in the meat shop and all eggs and dropping samples were collected from poultry farms in collection areas. All samples were collected in pre-sterilized stomacher bags (165 mm × 150 mm × 0.55 mm) in ice container with aseptic conditions and were transported to the microbiology department (centre of excellence Laboratory) of Primeasia University at the earliest convenience for processing and further assessment [23].

2.2 Isolation of Bacterial Cultures

After reaching of the samples to the laboratory for further analysis, 10 g of each sample were introduced to 90 ml of Buffered peptone water and incubated at 37°C for 24 hours for enrichment and 10ml of enriched samples were then transferred to 90ml of 7.5% Sodium Chloride broth for selective isolation of *S. aureus*, which was incubated again at 37°C. After incubation, a loopful of culture was streaked on Mannitol Salt Agar plate which was incubated in aerobic conditions at 37°C for 24 hours [24].

2.3 Identification of Bacterial Isolates

S. aureus was presumptively identified based on colony morphology of isolates on MSA agar. Golden yellow colonies formed on MSA agar surface were then stained using gram staining profiling. After staining, Hemolysis, 10% and 15% NaCl tolerance, Nitrate reductase test, Methyl Red test, Voges Proskauer test, Urease test, Coagulase, ONPG, Catalase, Oxidase test and utilization of Fructose, Glucose, Glycerol, Lactose, Mannose, Mannitol, Raffinose, Ribose, Salicin, Sucrose, and Xylose were performed. Binary matrice was developed based on positive results as 1 and negative results as 0. They were used to biotype isolates by calculating the similarity in complete linkage using STATA 14 software [25].

2.4 Antimicrobial susceptibility Testing

Kirby-Bauer disk diffusion method was performed using Mueller Hinton Agar to determine the antimicrobial activity of antibiotics against isolated *S. aureus* was measured *in vitro* [26]. By measuring the diameter of the zone of inhibition allowed the determination of the efficiency of an antibiotic that resulted from the diffusion of the antimicrobial agent into the medium surrounding the disc [23]. Strains were grown in Mueller Hinton broth overnight before inoculation on Mueller Hinton Agar plates. In this experiment, commercial antibiotic discs such as Tetracycline 30 μg (TE), Azithromycin 15 μg (AZM),

Table 1. Sample collection areas in a different location in Bangladesh

Name of collection area	Number of locations	Poultry meat	Egg	Dropping
Gazipur	6	6	6	6
Dhaka	7	7	7	7
Bogra	1	1	1	1
Narayanganj	2	2	2	2
Narsingdi	1	1	1	1
Pabna	4	4	4	4
Comilla	3	3	3	3
Jessore	1	1	1	1

**Every sample were collected triplicate

Sulphamethoxazole 25 µg (SXT), Ciprofloxacin 5 µg (CIP), Erythromycin 15 µg (E), Nalidixic Acid 30 µg (NA), Chloramphenicol 30 µg (C), Penicillin 10 µg (P), Colistin 10µg (COL), Meropenem 10 µg(MEM), Cefixime 15 µg (CFX), Chloramphenicol 30 µg (C) were used. The antibiotic discs were introduced on the agar surface by sterile forceps and incubated for 24hrs at 37°C. Post incubation clear zone of inhibition was observed and results were interpreted using Clinical and Laboratory Standards Institute (CLSI) guidelines [27].

2.5 Statistical Analysis

The experimental results were repeated triplicate each time and were subjected to analysis of variance (ANOVA). Means and standard deviations were calculated, while P-values were significant at the $P<0.05$ level and Dendrogram data were produced by STATA 14 Software [28].

3. RESULTS AND DISCUSSION

In this study, a total of 75 samples were collected, among them, 53 samples showed *S.aureus* isolates from chicken meat (18), egg (22) and dropping (13). The antibiotic-resistant pattern of the isolates was also observed (Fig. 1). Primary isolation of *S. aureus* was performed on MSA plates after enrichment in Buffered peptone broth and selective isolation in 7.5% NaCl broth by streak plate method. 75 samples were inoculated on MSA plates, 53(71%) of which exhibited yellow colonies due to positive D-Mannitol fermentation that indicated the presence of *S. aureus* on MSA. The remainder of the samples showed the presence of other organisms and therefore

excluded. Selected strains were sub-cultured and further characterized by biochemical typing. The numbers of isolates found in this study were slightly higher than the several isolates obtained from the previous another study [13].

Following isolation, strains were introduced to microscopy and biochemical test, results of which were used to generate a dendrogram to calculate the similarity between isolates based on binary matrice. Out of 53 isolates, 40 showed 100% similarity with *S. aureus* while the remaining 13 showed at least 86.5% similarity in a few cases (Fig. 2a, Fig. 2b and Fig. 2c). The dendrogram was generated to identify and find similarity between isolates of different origins (meat, egg & dropping) and locations. 4 branches were found in all sample types, where 40 isolates exhibited complete similarity (10 Dropping, 16 eggs and 14 meat isolates) with biochemical tests of *S. aureus*. Dendrogram can assist in the classification of the isolates based on their biochemical tests profiling and this similarity could be assessed using molecular techniques i.e. 16S rRNA gene sequence analysis.

Antibiotics have been used against infections since its discovery, however, in recent decade antibiotics are unable to act against infections properly due to the rise of drug resistance among pathogens. Unregulated and improper use of antibiotics contributes to the emergence of drug-resistant pathogens [29]. Therefore it is important to monitor the presence, control and disposes of such pathogens. It was seen that 24.52% (Azithromycin) to 86.62% (Penicillin) of the strains are resistant to the antibiotics used against them.
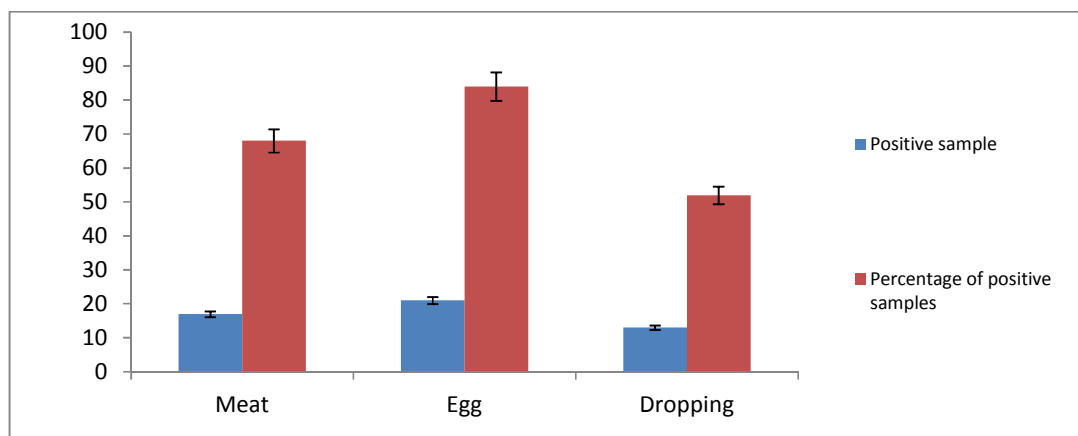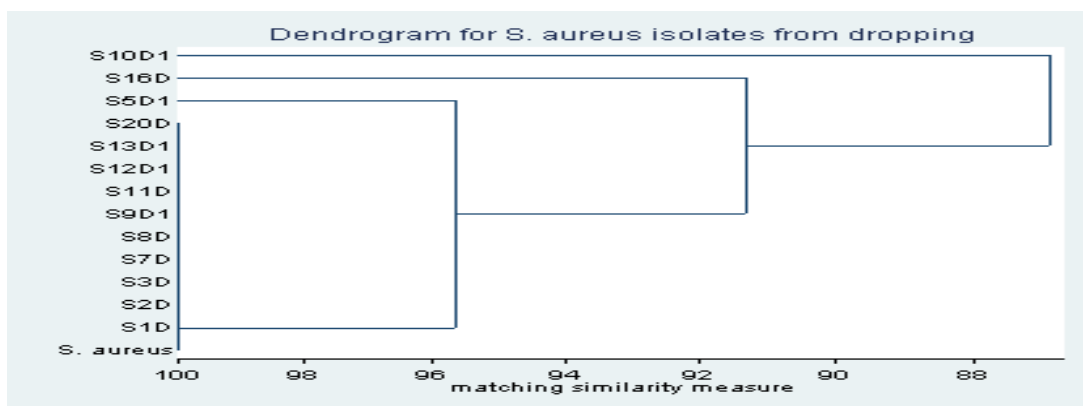

Fig. 1. Percentage of positive samples

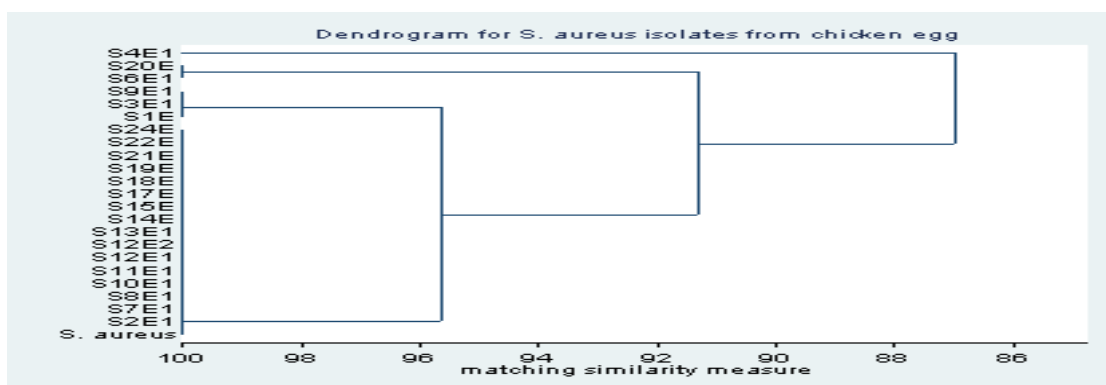**Fig. 2a. Dendrogram for dropping Isolates**

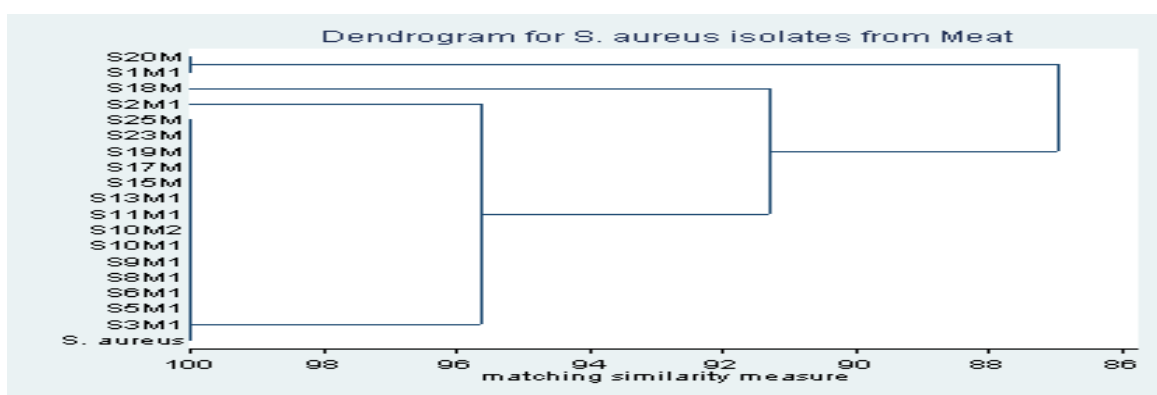**Fig. 2b. Dendrogram for egg isolates**

Fig. 2c. Dendrogram for meat isolates

Cefixime (62.23%) is the most sensitive drug against *S. aureus* isolates followed by Chloramphenicol (59.78%). In a study conducted in Taiwan, 50% isolates were found to be resistant to Tetracycline and 23.2% were resistant to Erythromycin which is similar to a current study, in fact, Erythromycin is more resistant [30]. Chloramphenicol exhibited similar resistance pattern to other studies conducted [31,32].

Chicken meat isolates (24.53%) exhibited the highest multi-drug resistant pattern, where the chicken egg and dropping *S. aureus* isolates

showed 18.89% and 15.04% multidrug-resistance pattern, respectively. Overall 58.49% isolates showed MDR against the test antibiotics. There were 12 isolates which were resistant against 5 drugs, 10 against 6 drugs and 8 against 7 drugs. Only one *S. aureus* isolate showed drug-resistance against 8 drugs. This data in the present study depicts a slightly higher number of MDR isolates in comparison to another study [24].

Microbiological quality and safety of food items have always been a matter of pressing concern. Poultry meat, especially chicken have widespread consumption rate worldwide due to its high availability and nutrition. But, often chicken meat and eggs are contaminated by various harmful pathogens, many of which are zoonotic such as *Salmonella* sp., *E. coli* and *Campylobacter* sp., while other pathogens may come from the environment or personnel related to slaughter, packaging, processing or retailing. Staphylococcal food-borne disease (SFD) is one of the most common food-borne illness-causing 241,000 cases annually in the USA [33]. Previous reports showed that 76.74% of chicken meat and 55% of egg samples showed the prevalence of *S. aureus* [20]. Our study also found that 68% of chicken meat and 84% of egg were infected. Multiple *S. aureus* outbreaks due to chicken meat, both raw and frozen, in Bangladesh, was previously recorded which varied from 24.56-95.83% [29,24,35]. We also found *S. aureus* infection in eggshell 84%, however, past data reported by authors from Bangladesh showed a higher prevalence of *S. aureus* from hen egg (66.67%) and duck egg (75%) in the local market [36,37]. Percentage of resistance of *S. aureus* isolated from our poultry meat samples to Penicillin, Erythromycin, Tetracycline, Sulphamethoxazole and Nalidixic Acid were found to be 88.89%, 61.11%, 55.56%, 55.56% and 50%, respectively. Datta et al. 2012 also found *S. aureus* resistance against penicillin and tetracycline 85.71% and 71.42% and another researcher from Bangladesh also found complete resistance to Erythromycin and Nalidixic Acid against *S. aureus* from poultry meat [35,2]. Data reported in several research articles from 2012 to 2017 showed no resistance to Ciprofloxacin among *S. aureus*, but in 2018, the isolates were 28.21% resistant against *S. aureus* from poultry meat [20]. In the current study, Ciprofloxacin was resistant 55.56% among meat isolates and 31.82% among egg isolates. *S. aureus* isolated from egg samples in our study was 86.36% resistant to penicillin, 68.18% resistant to Erythromycin, 36.36% and 31.82% resistant to Sulphamethoxazole and Ciprofloxacin. In the previous reports, 81.82% resistance against erythromycin, 72.73% against both ciprofloxacin and Sulphamethoxazole was observed. Azithromycin showed 90.91% sensitivity in the previous reports, while our report showed 77.28% sensitivity [20]. Meropenem (83.36%), Colistin and Cefixime were both 81.82% sensitive in our study.
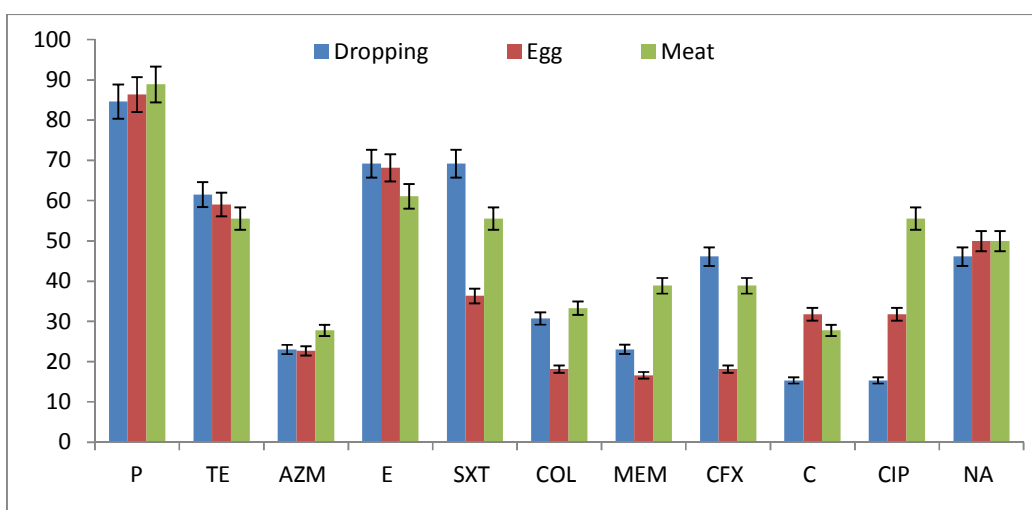

Fig. 3. Comparative analysis of resistance (%) of *S. aureus* to antibiotics
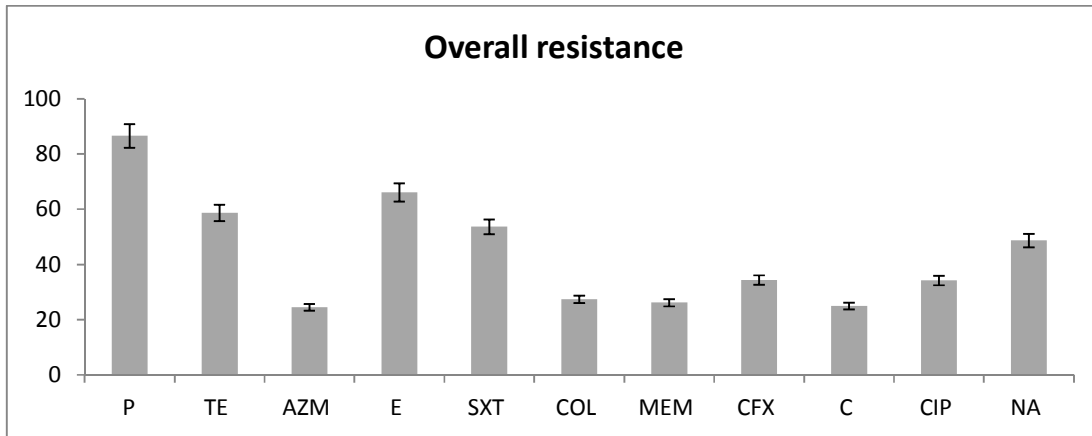
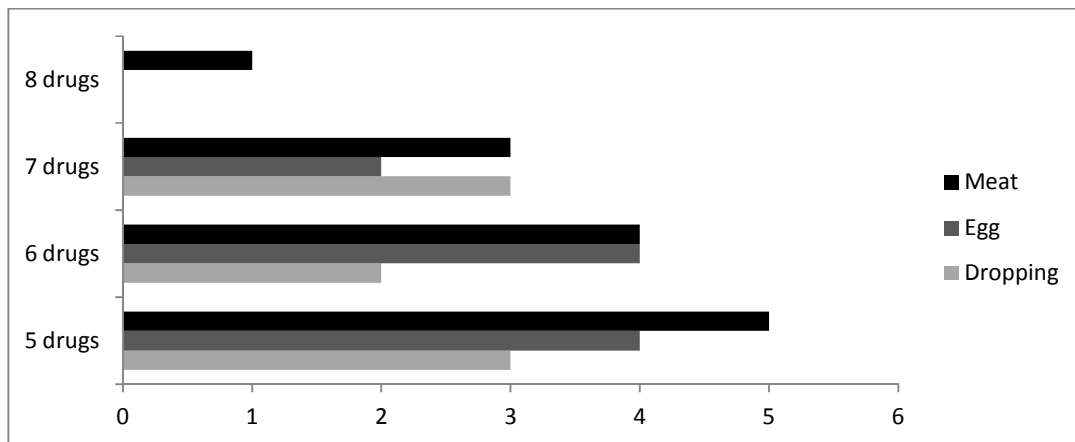**Fig. 4. Overall resistances (%) of *S. aureus* to each antibiotic**

Fig. 5. Multi-drug resistance pattern of *S. aureus*

However, despite high prevalence, the low reported annual incidence is caused by the unwillingness of patients seeking medical attention, misdiagnosis, improper sample collection and processing, poor routine surveillance of *S. aureus* and enterotoxins in samples [34]. This study is hoped to make people aware of the presence of drug-resistant *S. aureus* in meat and egg, which may cause serious concern to public health if left unsupervised.

4. CONCLUSION

This study is not a new phenomenon which has been corroborated by various investigators. Poor hygiene, undercooked or raw meat and egg, improperly disposed poultry litter into the environment may increase migration risk of this drug-resistant *S. aureus* into food, environment, and people related to the poultry industry.

Moreover, similar to other countries, Bangladesh is also facing the emergence of drug-resistant clinical pathogens. Drug-resistant *S. aureus* which causes food poisoning may assist in transferring drug-resistant mechanism to clinical isolates making the situation worse. Periodic supervision and proper analysis should be conducted regarding the drug-resistant isolates to control infection, intoxication, contamination, and transfer of drug-resistant mechanism from meat, egg and dropping to humans.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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