ANTIBIOGRAM OF PRE-PROCESSED RAW CHICKEN MEAT FROM DIFFERENT SUPERSHOPS OF DHAKA CITY, BANGLADESH

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Abstract: Pathogenic strains of salmonella, S. aureus, S. epidermidis, shigella, enterobacter and citrobacter are serious health threat for human being. Present investigation was conducted to assess the antibiogram of pre-processed raw chicken meat collected from different chain-shops of Dhaka city. Pre-processed chicken meat samples were collected from three different super-shops (S1, S2, and S3) in Dhaka. The Samples are serially diluted to 10⁴, and after growth on MSA, MAC, and SS agars, the total counts showed The most growth S2 (360 \times 10.4 \pm 18.33), (235 \times 10.4), $(25 \times 10.14.4 \pm 3.6)$ followed by S3 $(353.3 \times 10.4 \pm 42.39)$, $(151.67 \times 10.4 \pm$ 22.19), 15×10 -4 ± 3.0) and finally S1 (3.26.67 \times 10.4 \pm 29.48), (64.5 \times 10.4 \pm 8.62) (14 \times 10.4 \pm 5.13) respectively. Salmonella S aureus, S. epidermidis, Shigella, Enterobacter and Citrobacter were identified after a number of morphological and biochemical tests; Gram staining, MIU, KIA, oxidase and catalase tests, and citrate utilization test following aseptic techniques. Six antibiotics; Vancomycin, and five broad spectrum drugs; Streptomycin, Norfloxacin, Novobiocin, Chloramphenicol, and Cefotaxime were used to test the sensitivity or resistance of the organisms. All the pathogens were susceptible to chloramphenicol. Staphylococcusepidermidis were novobiocin-resistant. Citrobacter spp. and Enterobacter spp. were sensitive to all except novobiocin. Staphylococcusepidermidis was susceptible and intermediate to norfloxacin. Staphylococcusaureus was resistant to norfloxacin and novobiocin; intermediate to cefotaxime, and sensitive to the rest. Salmonella spp. was found to be resistant to novobiocin, vancomycin, and streptomycin; intermediate to norfloxacin, but sensitive to chloramphenicol and cefotaxime. Shigella.spp was found to be sensitive to all the antibiotics but resistant to novobiocin and vancomycin. Fecal coliform E. coli was absent showing some degree of sanitation in the chicken, and all the pathogens were found susceptible to at least two antibiotics.

Keywords: Chicken, morphological, antibiogram, chloramphenicol, Shigella spp, novobiocin.

Introduction

Recently, food safety has become an extremely important issue linking with public health in Bangladesh. Poultry sub-sector is one of the important areas associated with food safety issue. Chicken is the most widely recognized sort of poultry in the world and is

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ready as sustenance in such a variety of distinctive courses, varying between societies and religions. Different sorts of chicken, for example, pullets, capons, and hens are consumed over a large portion of the Eastern half of the globe. Chicken serves as a great wellspring of lean, top notch protein; that is, protein that holds the eight crucial amino acids and a moderately low measure of fat. Besides the fat present in chicken is basically of the unsaturated sort, which secures the body against coronary illness¹.

Poultry meat includes about 20 to 23% protein, and its comminuted items, for example, hotdogs, bologna and wieners ordinarily hold something like 17to 20% protein, 0 to 20% fat, and 60 to 80% water². Protein is a key supplement in human development and improvement and it likewise assumes a paramount part in aiding individuals who are overweight and stout in getting thinner. It is likewise rich in vitamin B6 that is essential in the human invulnerable framework and the support of glucose level. Zinc held in chickened meat is critical in the working of the invulnerable framework and DNA union.

Microbiological foodborne ailments are created by different microbes or their metabolites, parasites or their poisons and so forth. The vitality of nourishment borne infections relies on upon sustenance handling framework, taking care of capacity condition, affectability of the populace and the kind of buyers. Meat is exceptionally powerless to decay, as well as habitually embroiled in the spread of nourishment borne ailment. The defiled crude meat is one of the primary wellsprings of foodborne disease. Throughout the transformation of butchers and transforming, all possibly edible tissues are subjected to defilement from an assortment of sources inside and outside creature. In living creatures, those surfaces in contact with nature's domain harbor a mixture of microorganisms. The polluting organic entities are inferred essentially from the stow away of the creature and additionally involve life forms that begin from both excrement. What's more, handled meat sustenance's are more inclined to pollution with pathogenic microorganisms throughout the different phases of preparing. Meat and meat items are vital wellsprings of human contaminations with an assortment of foodborne pathogens, i.e. Salmonella spp., verotoxigenic, Escherichia coli, Pathogen in meat eg. Salmonella spp. is most proficiently controlled by the principle mediations connected in the essential handling consolidated with the streamlining of the butcher cleanliness. The aimless utilization of antimicrobial medications in nourishment creatures may bring about change of imperviousness in human body, it is improbable that the purported oppositeantimicrobial pill will be limited to use in human prescription³. The issue may be because of the characteristic safety of species to specific anti-infection agents, conceivable exchange of anti-toxins safety around species and the utilization of sub therapeutic measurements of anti-microbial in creature nourish to enhance creature benefit could likewise select for safety strains. Lately, an emotional build in the safety against antiinfection agents routinely utilized as a part of human and also in veterinary prescription has been recorded in the parts of the family Staphylococcus. Despite the fact, it is not certain to demonstrate an immediate part of medication safety in microbes tainting nourishment things with expanded clinical instances of safe diseases, the vicinity of such microscopic organisms in sustenance things and their related surroundings could assume a part on the spread of antimicrobial safety around nourishment borne pathogens⁴. This study endeavors to survey the clean measures of preprocessed chicken meat sold at diverse super-shops in Dhaka city. It ought to help in the refinement of customers who buy such chicken meat and give knowledge of their microbiological quality

Materials and methods

This study was done in the Microbiology Laboratory of Department of Biology and Chemistry, 4th Floor, North South University. Pre-processed chicken meat samples were gotten from three different super-shops in Dhaka; Market 1, Bashundhara Residential Area; Market 2, Gulshan 2 and Market 3, Gulshan 2. These were carefully collected in an autoclaved container and moved to the laboratory.

Sample Preparation

Ten grams of each sample were measured with a weighing balance, following proper aseptic technique and were transferred to separate sterile beaker containing ninety ml sterile distilled water to make 10⁻¹ dilution and shacked well with vortex mixer. Then 1 ml form that dilution was transferred to 9 ml distilled water to get the 10⁻² dilution and the dilution continued in this series until 10⁻⁵. Diluted meat samples in distilled water were spread onto MacConkey agar (MAC), Salmonella Shigella (SS) agar, and Mannitol Salt Agar (MSA) plates and incubated at 37°C for 24 hr. Bacterial isolates were confirmed by microscopic, cultural and standard biochemical tests (motility, catalase, oxidase, urease, citrate utilization, indole, KIA test) according to Bergey's Manual of Determinative Bacteriology, 9th Edition, 1994 for further analysis.

Antibiotic Sensitivity Test

This was done using the Kirby-Bauer method by standard disc-diffusion method. Overnight grown cultures were used for the test. To test the sensitivity or resistance of the different microorganisms to some commonly used antibiotics; Vancomycin (VA) $30\mu g$, and broad spectrum drugs; Norfloxacin (NOR) $10\mu g$, Streptomycin (S) $10\mu g$, Chloramphenicol C $30\mu g$, Novobiocin (NV) $30\mu g$, and Cefotaxime (CTX) $30\mu g$ (Kirby–Bauer, 1997).

Results

Table 1: Total Plate Count Results

Sample	Place of sample	Media	Dilution	Count	Mean ±
	collection		factor	Results	SD
				CFU/ml	
S1A	Market 1(Bashundhara)			304 x 10	326.67 x
S1B	Market 1(Bashundhara)	MSA	10^{-4}	316 x 10	$10^{-4} \pm 9.48$
S1C	Market 1(Bashundhara)			360 x 10	
S1A	Market 1(Bashundhara)			67 x 10 ⁻⁴	64.5 x 10-4
S1B	Market 1(Bashundhara)	MAC	10^{-4}	73 x 10	± 8.62
S1C	Market 1(Bashundhara)			56 x 10	
S1A	Market 1(Bashundhara)			12 x 10	14 x 10-4 ±
S1B	Market 1(Bashundhara)	SS	10^{-4}	9 x 10	5.13
S1C	Market 1(Bashundhara)			19 x 10	
S2A	Market 2 (Gulshan 2)			336 x 10	360 x 10-4
S2B	Market 2(Gulshan 2)	MSA	10^{-4}	372 x 10	± 18.33
S2C	Market 2(Gulshan 2)			348 x 10	
S2A	Market 2(Gulshan 2)		10^{-4}	256 x 10	235 x 10-4
S2B	Market 2(Gulshan 2)	MAC		242 x 10	± 14
S2C	Market 2(Gulshan 2)			228 x 10	
S2A	Market 2(Gulshan 2)			12 x 10	25 x 10-4 ±
S2B	Market 2(Gulshan 2)	SS	10^{-4}	9 x 10	3.6
S2C	Market 2(Gulshan 2)			19 x 10	
S3A	Market 3(Gulshan 2)			308 x 10	353.3 x 10-4
S3B	Market 3(Gulshan 2)	MSA	10^{-4}	360 x 10	± 42.39
S3C	Market 3(Gulshan 2)			392 x 10	
S3A	Market 3(Gulshan 2)		10 ⁻⁴	155 x 10	151.67 x
S3B	Market 3(Gulshan 2)	MAC		172 x 10	10-4 ±
S3C	Market 3(Gulshan 2)			128 x 10	22.19
S3A	Market 3(Gulshan 2)		10-4	15 x 10	15 x 10-4 ±
S3B	Market 3(Gulshan 2)	SS		12 x 10	3.0
S3C	Market 3(Gulshan 2)			18 x 10	•

S1: Sample 1, S2: Sample 2, S3: Sample 3, MSA: Mannitol Salt Agar, MAC: MAC Conkey Agar, SS: Salmonella/Shigella Agar,

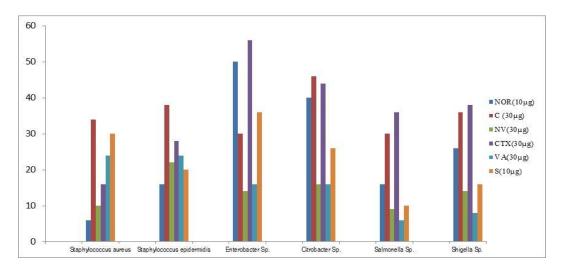


Figure 1: Antibiotic sensitivity test results for S1

NOR: Norfloxacin, C: Ciprofloxacin, NV: Novobiocin, CTX: Cefotaxime , VA: Vancomycin,

S: Streptomycin

*S: Sensitive, R: Resistant, I: Intermediate

In Sample 1 (S1) the antibiotic sensitivity of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Citrobacter spp.*, *Salmonella spp.*, *Shigela spp.*, *Enterobacter spp.* were shown (Chart 1). CTX and NOR has good sensitivity in *Enterobacter spp.* and *Citrobacter spp.*, C has been found good sensitivity in *Staphylococcus aureus* and *Staphylococcus epidermidis*. In case of *Salmonella spp.* and *Shigellaspp.* CTX has been found most effective than other drugs.

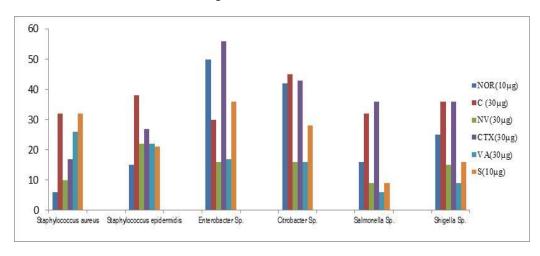


Figure 2: Antibiotic sensitivity test results for S2

In Sample 2 (S2), C and S are effective drug against *Staphylococcus aureus*. For *Staphylococcus epidermidis* C is the most effective, for *Enterobacter spp*. CTX and NOR havebeen found very good sensitive. In *Citrobacter spp*. NOR, C and CTX were most sensitive drug. C and CTX were good against *Salmonella spp*. and NOR, C and CTX were found very good against *Shigella spp*. (Chart 2).

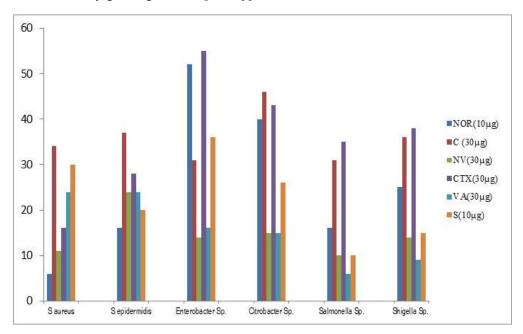


Figure 3: Antibiotic sensitivity test results for S3

In Sample 3 (S3), C and S are most effective drug against *Staphylococcus aureus*. For *Staphylococcus epidermidis* C is the most effective, for *Enterobacter spp*. CTX and NOR havebeen found very good sensitive. In *Citrobacter spp*. NOR, C and CTX were most sensitive drug. C and CTX were good against *Salmonella spp*. and NOR, C and CTX were found very good against *Shigella spp*. (Chart 3).

Discussion

Chicken meat is an extremely nutritious meat item and it is consumed by individuals of all ages, throughout the world¹. In developing countries, for example, Bangladesh, the eating methodology of individuals living in urban communities generally holds more protein from living beings than the eating methodology of individuals from the provincial locales. This could essentially be on the grounds that the urban individuals are more prosperous, additionally in light of the fact that they for the most part have an entry to a more extensive assortment of nourishments at the neighbouring markets. Notwithstanding its wellbeing points of interest, if not took care of carefully, chicken meat might be less than great.

In this study, the preprocessed chicken meat specimens were utilized, inferring some level of clean measures were actualized⁶, and reported abnormal amounts of microbiological pollution and high frequency rates with E. coli in retail chicken. An alternate report from indicated that E. coli was found in the greater part of the retail chicken remains and their items that were tried⁸ in spite of the fact that recommended that while the destruction procedure brought about a noteworthy expand in microbial fecal sullying, easier microbial include were watched hygienically handled bodies as contrasted and business sector remains, and that E. coli was totally missing in hygienically transformed remains. Owing to the way that the fecal coliform E. coli was non attendant in the majority of the preprocessed chicken specimens, shows generally great hygienic states of the super-shops. Notwithstanding the preprocessed chicken meat examples were not totally without tainting Actually, the vicinity of coliforms, for example, Citrobacter spp. what's more Enterobacter spp. and additionally other sustenance-borne pathogens; Salmonella, Shigella, Staphylococcus, demonstrates sullying of the chicken examples, and plausibility of them to cause nourishment-borne ailments. The differences in microbial growths too were found amongst the different samples gotten from the three super-shops S2 (360 \times 10.4 \pm 18.33), (235 \times 10.4), (25 \times $10.14.4 \pm 3.6$) followed by S3 (353.3 × 10.4 ± 42.39), (151.67 × 10.4 ± 22.19), 15 × 10-4 ± 3.0) and finally S1 (3.26.67 \times 10.4 \pm 29.48), (64.5 \times 10.4 \pm 8.62) (14 \times 10.4 \pm 5.13) respectively. Salmonella Since all the samples were processed in the same way, the difference in total viable counts can therefore be attributed to individual contamination by the handlers; causing a higher load in some than others; with S2 most contaminated, followed by S3 and S1 as the least contaminated. Two species of Staphylococcus were found in the chicken meat samples; S. aureus, and S. epidermidis. After the antibiotic sensitivity testing, S. aureus was found to be novobiocin resistant, while S. epidermidis was found to be susceptible to novobiocin. S. aureues was resistant to norfloxacin, while S. epidermidis was intermediate; S aureus was then found to be intermediate to Cefotaxime while S. epidermidis was Cefotaxime-resistant. They were both susceptible to Choramphenicol, Streptomycin and Vancomycin. The susceptibility of S aureus to Vancomycin is expected as Vancomycin continues to be used as a first-line antimicrobial agent for the treatment of infection with methicillin-resistant Staphylococcus aureus (MRSA). Unfortunately, Vancomycin-resistant S aureus (VRSA) infections, although rare (about seven cases reported in the USA) are surfacing, and are a big public health problem⁹. All the pathogens were found to be susceptible to chloramphenicol. This shows that chloramphenicol is a good broad spectrum drug, and inhibits a large amount of bacterial growth. All the pathogens except S epidermidis were found to be resistant to novobiocin. In the treatment of food-borne illnesses, chloramphenicol should therefore have a higher preference over novobiocin. Citrobacter spp. and Enterobacter spp. were sensitive to all except novobiocin. S. epidermidis was susceptible and intermediate to norfloxacin. S. aureus was resistant to norfloxacin and novobiocin: intermediate to cefotaxime, and sensitive to the rest. Salmonella spp. was found to be resistant to novobiocin, vancomycin, and streptomycin; intermediate to norfloxacin, but sensitive to chloramphenicol and cefotaxime. Shigella spp. was found to be sensitive to all the antibiotics but resistant to novobiocin and vancomycin.

Conclusion

The unlucky deficiency of the fecal coliform *E. coli*, which is utilized as a marker of clean conditions, and determinant of fecal sullying, demonstrates some level of sanitation and cleanliness on the way of the preprocessed chicken meat suppliers in Dhaka city. The vicinity of the other nourishment borne microorganisms; *Salmonella spp., Shigella spp., Enterobacter spp.*, *S. aureus, S. epidermidis, Citrobacter spp.*, demonstrates that the chicken meat examples were not precisely perfect. These organic entities in the examples can result in sustenance-borne sicknesses, for example, Shigellosis, Salmonellosis, the runs, etc. These pathogens can without much of a stretch be hindered by some basic antimicrobials, as indicated in the outcomes, each of the pathogens were helpless to no less than two anti-microbials. Chloramphenicol showed the highest zones of inhibition, and all the microorganisms were susceptible to it, hence it should be preferred over novobiocin which showed resistance in all but *S. epidermidis*, in the treatment of foodborne illnesses involving these pathogens.

References

- National Chicken Council..http://www.nationalchickencouncil.org/wp-content/uploads/2012/08/2012-NCC-Consumer-Survey.pdf.
- 2. Al-Dughayma A, Altabari GF, Hamdy M. Safety and Quality of Chicken Meat Products. (http://www.kfu.edu.sa/ar/Deans/Research/Documents/3003.pdf).
- 3. Schwarz S, Chaslus DE. Use of antimicrobials in veterinary medicine and mechanism if resistance. 2001., Vet Res. 32, 201-225.
- 4. Datta S, Akter A, Shah IG, Shah K, Fatema TH, Islam., Bandyopadhyay Z, Khan UM, Biswas D. Microbiological Quality Assessment of Raw Meat and Meat Products, and Antibiotic Susceptibility of Isolated Staphylococcus aureus, Agric. Food Anal. Bacteriol. 2012.,2, 187-194.
- Holt JG, Kreig NR. Bergey's Manual of Determinative Bacteriology, 9th Edition. Lippincott Williams & Wilkins. MI, USA. 1994.
- 6. Alvarez-Astorga M, Capita R, Alonso-Calleja C, Moreno B, Garcia-Fernandez M.C. Microbiological quality of retail chicken by-products in Spain. Meat Sci. 2002. 62, 45-50.
- 7. Vural A, Erkan ME, Yeilmen S. Microbiological quality of retail chicken carcasses and their products in Turkey. Med. Weter. 2006, 62, 1371-1374.
- 8. Yashoda KP, Sachindra NM, Sakhare PZ, Rao DN. Microbiological quality of broiler chicken carcasses processed hygienically in a small scale poultry processing unit. J. Food Quality. 2001, 24, 249–259.
- 9. Finks J, Wells E, Dyke TL, Husain N, Plizga L, Heddurshetti R. Vancomycin-resistant Staphylococcus aureus, Michigan, USA. Emerg Infect Dis [serial on the Internet]. 2009 Jun [date cited].http://wwwnc.cdc.gov/eid/article/15/6/08-1312.htm
- 10. Kirby-Bauer Method. Disk Diffusion Susceptibility Testing. Newsletter of Animal Disease Diagnostic Laboratory. http://www.addl.purdue.edu/newsletters/1997/spring/dds.shtml.
- 11. Center for Food Safety and Applied Nutrition of the Food and Drug Administration (FDA), U.S. Department of Health and Human Services. Bad Bug Book Foodborne Pathogenic Microorganisms and Natural Toxins Handbook, 2012. 2nd Ed.