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Research paper

Prevalence of Antibiotic Resistant E.Coli and Salmonella from Retail Poultry Meat across Mumbai.

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Abstract

Salmonellosis and Escherichia coli infection is a major public health concern both in developed and developing countries. The use of antimicrobial therapy is the main mode of treatment for infections caused by E.coli and Salmonella spp . The majority of these infections are associated with the consumption of products such as poultry, meat, water, vegetables, eggs, milk, seafood, and fresh produce contaminated with Salmonella and E.coli. The objective of this study was to check the prevalence of antibiotic resistant E.coli and Salmonella spp from freshly cut raw poultry meat and to know the antimicrobial sensitivity pattern of these isolates to common antibiotics. Total number of 50 retail poultry meat samples was studied from different locations across Mumbai. All samples were randomly selected from each ward and all the samples were found to be contaminated with E. coli and Salmonella spp. Out of 50 samples all of them showed the presence of E.coli and salmonella .103 E.coli isolates and 282 Salmonella isolates were identified. The E. coli and Salmonella isolates were found to be resistant to multiple antibiotics including Ampicillin, Gentamicin, Vancomycin, Tobramycin, Streptomycin, Ciprofloxacin and Ceftriaxone. Using the CLSI 2015 breakpoints for disc diffusion, 0.97% (1/103) E.coli isolates and 1.42% (4/282) Salmonella isolates were intermediately resistant to Ceftriaxone and the remaining isolates were resistant to all the other antibiotics used in the study. This study highlights the urgent need for surveillance and check on the rapid spread of antibiotic resistance via the food borne pathogen route to the human population.

Keywords: *antibiotic resistance, E.coli, raw poultry, Salmonella.*

1. Introduction

According to the WHO, E. coli and Salmonella are the most common and frequent pathogens responsible for food poisoning and food related infections and Escherichia coli alone is responsible for 25% of the infant diarrhoea in developing countries .E. coli O157:H7 as an important cause of bacterial diarrhoea, and that the spectrum of illnesses caused by this pathogen include asymptomatic carriage, nonbloody diarrhoea, haemorrahgic colitis, thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome[1].Also an E.coli O157:H7 outbreak resulted in 21 people hospitalized and 3 cases of hemolytic uremic syndrome (HUS) and illness in at least 61 persons [2].E.coli inhabit the gut of human and animal, also it is common flora of the gut and voided in faeces and also remain viable for few days into the environment and therefore could be used as an indicator for recent faecal contamination of food products. Studies have soon some modes which could transmit E. coli O157:H7 to humans may include direct or indirect association with cattle and bovine derived vehicles include raw milk, cheese and butter [3], unpasteurized milk. Produce items associated with outbreaks include spinach, lettuces, apple cider and juice, coleslaw, and sprouts [3] both drinking water and recreational waters have been the sources of E. coli O157:H7 that caused human illness outbreaks [4]. E. coli O157:H7 outbreak has also been associated with restaurant that ground locallyproduced beef for hamburgers on site [5]. Salmonella is among the leading cause of food borne illness a Salmonellae can enter and survive in the farm environment for long periods of time. Prevalence of Salmonella spps in the environment of farm ranges from 10 to 26% and presence of Salmonella in feed and feed ingredients is also highlighted into recent studies. However, very low levels of Salmonella have been obtained from drinking water samples from broiler farms. Conversely, recovery of Salmonella was easily accomplished in samples from standing water where the bacteria can persist in biofilm formation. Shell eggs are a most common vehicle for S. enteritidis in humans leading to Salmonellosis a major outbreak occurred in 1994 where it was reported that previously carried S. enteritidis contaminated liquid eggs cause the cross contamination of ice-cream[6].Serovar Enteritidis is known to be very well adapted to the hen house environment, the bird, and the egg. Most commonly, hens are infected with S. enteritidis by vertical transmission and through transovarian infection eggs may become contaminated. Due to the rapid and widespread emergence of S. typhi serotypes with resistance to multiple antibiotics and also changing modes of bacterial presentation, typhoid fever is becoming one of the increasingly difficult to diagnose and treat parasite[7].The emergence of multidrug-resistant strains of S. typhi and E. coli has made it necessary to develop more effective antibiotics and vaccines . However despite recent advances in antibiotics research a lot more needs to be done to mitigate the antibiotics problem with better understanding and surveillance. Studies world over indicate a need of continuous surveillance and sharing of antimicrobial susceptibility data for Salmonella and E. coli among countries

worldwide since the organism has been gaining resistance at a higher rate also to ensure the effectiveness of control programmes.

2. Materials and Methods

All media and Antibiotic discs used in the study were obtained from Hi media laboratories ,Mumbai, India and the chemicals used in the study were of analytical grade obtained from local manufacturers.

a. Sampling: A total of 50 samples of freshly cut chicken was collected aseptically from the local poultry shops across Mumbai city ,Maharashtra , India. Approximately 50 grams of chicken samples was collected into a sterile plastic container and was transported to laboratory in an ice box container and was processed within 2 hours of collection.

b. Total plate count : The total plate count of the chicken samples were carried out as soon as the arrived in the laboratory using plate count agar by serial dilution methods

c . Isolation of Salmonella: Salmonella was isolated by the conventional method recommended by FDA and Andrew 1996. The pre enrichment was carried out by inoculating 25 gm of chicken in 225 ml of peptone water and was incubated for 37°C for 24 hours. For enrichment of salmonella 1 ml of the pre enrichment broth was inoculated into 9 ml of selenite cystine broth aseptically and was incubated at 37°C/24 hours. Then a loopful of the enrichment media as streaked onto the XLD (xylose lysine deoxycholate agar) plates in duplicate incubated at 37°C for 24 hours .Suspected colonies of Salmonella (minimum of five to eight colonies) from each selective agar plate were subjected to biochemical tests which includes catalase test, TSIA (triple sugar iron agar test), citrate test confirmation antimicrobial sensitivity was performed .

d. Isolation of E.coli: For the enrichment of E.coli 1 ml of the pre enrichment broth was inoculated into 9 ml of MacConkey broth aseptically and was incubated at 37°C/24 hours. After incubation a loopful of the MacConkey broth culture was streaked on MacConkey agar plates in duplicate and as incubated at 37°C for 24 hours. Pink colonies obtained after incubation (minimum of five to 8 colonies) on the MacConkey agar plates was then subjected to biochemical tesst IMViC i.e. Indole ,Methyl red, Vogues -Proskauer test, Citrate), TSIA (triple sugar iron agar test).After confirmed as E.coli the isolates were subjected to antimicrobial susceptibility testing

e. Antibiotic sensitivity: The antimicrobial susceptibility testing was carried out using the Kirby-Bauer disk diffusion technique .The media used for testing was Muller Hinton (MH) agar. The antibiotics were placed aseptically on to the MH agar and were incubated at 37°C for 16-18 hours and zone of inhibition around the antibiotics was measured. Clinical and Laboratory Standards Institute (CLSI) 2015 guidelines were used to interpret results [16].The following antibiotics were used: ampicillin(AMP,10mcg), vancomycin (VA, 30mcg), gentamicin (GEN,10mcg), ciprofloxacin (CIP,5mcg), rifampicia (RIF, 5mcg), tobramycin(TOB, 10mcg), streptomycin (S,10mcg), ceftriaxone (CTR,30mcg) antibiotics were manufactured by Himedia (Mumbai, India)

3. Results

The total aerobic viable counts obtained from samples across the different wards in the city ranged from log values 6.7 to 3.6per gram of sample .Indole positive E.coli isolates ranged from log values 2.30 to 1.2 and Salmonellae ranged from log values 2.47 to 1. As given in the Figure 1 and 2 below highest percentage of antibiotic resistant isolates were obtained from the eastern suburbs of Mumbai

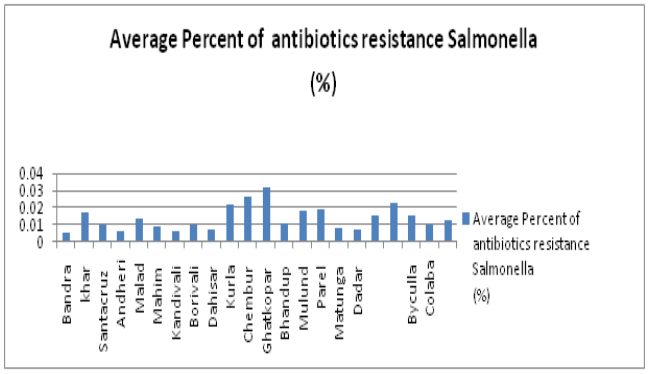

Fig 1: Average antibiotic resistant Salmonella (%) isolates from different locations across Mumbai

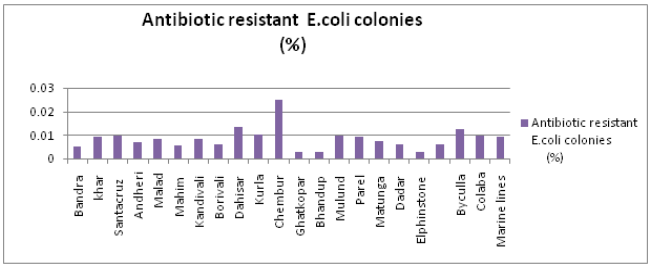

Fig 2: Average antibiotic resistant E.coli (%) isolated from different locations across Mumbai

Discussion : A number of studies from different parts of the world including Thailand (8) Qatar (9) Bangladesh (10)and northern India(11) have highlighted the prevalence of antibiotic resistance in isolates of E.coli and Salmonella in poultry meat and other meats indicating unhygienic veterinary practices followed indicators of heavy load of antibiotics in poultry. Our results also corroborate these studies and highlight a crying need to address this important food safety issue. We found almost all isolates to be resistant to the antibiotics tested 0.97% (1/103) E.coli isolates and 1.42% (4/282) Salmonella isolates were intermediately resistant to Ceftriaxone.

4. Conclusion:

The findings of our study provide evidence to suggest the existence of a reservoir of antibiotic resistance genes in poultry also infections with multidrug-resistant pathogens limit the options available to treat infectious disease of animals and humans. The high prevalence of multidrug-resistant E.coli and Salmonella observed in this study, mainly suggests there is a need for creating awareness on judicious use of antibiotics , dosage pattern and also to improve education and communication on the issue of antibiotic use in various human and veterinary medicine. Finally, we hope that the results obtained will make a positive impact in the field of public health.

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