ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* ISOLATES FROM CHICKEN MEAT SAMPLES IN DUBAI, UNITED ARAB EMIRATES

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Abstract: The objective of this study was to isolate and verify the sensitivity to antimicrobial agents of strains of *Salmonella* spp. isolated from chicken meat samples in Dubai, United Arab Emirates. A total number of 60 fresh chicken meat samples mainly chicken breast, chicken wing and chicken thigh were randomly collected from various supermarkets and butcheries in Dubai. The methods for *Salmonella* isolation and characterisation along with the susceptibility of these isolates to antibiotics were assessed. In total 66 pure isolates of Salmonella were obtained. The genus level identification of these isolates was carried out by Fluorescence In Situ Hybridisation (FISH) using the VIT-Salmonella kit (Vermicon Identification Technology, Vermicon, Munich, Germany). All the isolates tested positive for Salmonella genus specific oligonucleotide probe. These isolates were then subjected to antimicrobial susceptibility testing to 10 commonly used antibiotics. All the

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isolates (100%) showed resistance to Cephalexin and Rifampicin. A high degree of resistance was also observed for Ampicillin and Tetracycline while 87.88% of these isolates were sensitive to Ciprofloxacin and Amikacin. The prevalence of *Salmonella* strains resistant to more than one antibiotics may be due to the comprehensive use of antibiotics included in feeds as growth promoters and due to the widespread use of antibiotics in poultry industries. In conclusion, a high degree of multiple drug resistance was observed in *Salmonella* isolates from chicken meat samples that indicate that drug resistance of *Salmonella* is becoming a crucial health problem in this part of the world.

Keywords: Salmonella; antibiotic resistance; chicken meat, fluorescence in situ hybridisation; FISH.

INTRODUCTION

Salmonella typhimurium has been recognised worldwide as the major causes of most cases of human salmonellosis transmitted through food, water and other environmental sources (Baggesen et al., 2000; Baudart et al., 2000; Carlson et al., 1999; Humphrey 2000; Sharma and Carlson, 2000). Each year, approximately 40,000 *Salmonella* infections are culture-confirmed, serotyped and reported to the USA Centers for Disease Control and Prevention (CDC), which estimates an annual rate of 1.4 million cases, 16,430 hospitalisations and 582 deaths in the USA alone (Mead et al., 1999). Of total cases, 95% are estimated to be caused by foods. The total annual cost resulting from food borne Salmonella infections in USA is estimated in about US$3.5 billion (Pang et al., 1995). Hence, Salmonellae are considered to be potential pathogens having economic significance the world over. International data summarised by Thorns (2000) provides estimated incidences of Salmonellosis per 100,000 people for the year 1997: 14 in the USA, 38 in Australia and 73 in Japan. In the European Union 192,703 cases of Salmonellosis were reported in 2004 which represents an incidence of 42.2 per 100,000 people. Incidence ranged from 6.6/100,000 people in Portugal to 300.9/100,000 in the Czech Republic (EFSA, 2006).

The importance of Salmonella in the public health is very significant; for instance, it accounted for nearly 84% of food-borne human illnesses in Scotland from 1980 to 1989 and 81% in Italy from 1991 to 1994 (Oliveira et al., 2006). In 2006, the latest figures available from the Dubai Department of Health and Medical Services (DOHMS), showed there were 54 cases of salmonella food poisoning, comprising 0.8% of all reported infectious disease cases. From the early 1990s, the incidence and severity of human Salmonellosis cases has been increasing, presumably due to a rise in antimicrobial resistant *Salmonella* spp. strains. Strains with broad antimicrobial resistance are becoming common, for antimicrobials commonly used in animal husbandry as well as in medicine. In a recent study of *Salmonella* spp. isolates in the United Arab Emirates and Kuwait, 4.1 and 9.8% of isolates were found to be multidrug resistant respectively (Rotimi et al., 2008). Of the nine antibiotics studied, resistance was most developed against ampicillin and trimethoprim-sulfamethoxazole. Reduced susceptibility to ciprofloxacin was observed in 14.2% and 7.4% of the non-typhoidal *Salmonella*, respectively, as were in 44% of *Salmonella* enterica serovar *typhi* and 66.7% *Salmonella paratyphi*. The emergence of quinolone resistant *Salmonella* spp. in these countries could compromise medical treatment. As there is no documented study of prevalence of *Salmonella* sp. in food products available in the United Arab Emirates, this study was carried out to evaluate the pervasiveness of Salmonella sp. in fresh

chicken meat samples as poultry are often incriminated as reservoirs of *Salmonella* sp. For effective long-term control of *Salmonella* infection, assessment of safety and quality of food is important.

The aim of this study was to isolate and identify *Salmonella* spp. strains from Dubai food retail outlets, evaluate their prevalence on fresh chicken samples, and determine their resistance profile to ten commonly used antimicrobial agents. Fluorescence *in situ* Hybridisation (FISH) was used for *Salmonella* genus identification, and the technique was evaluated for effectiveness in food screening.

MATERIALS AND METHODS

Sample collection

About 60 fresh chicken meat samples mainly chicken breast, chicken wing and chicken thigh were randomly collected from various supermarkets and butcheries in Dubai, UAE. This random sampling included most of the imported chicken brands in super markets and butcheries in Dubai. Samples were taken from February to May 2009, spanning late summer to early winter. Samples were refrigerated after purchase, transported to the laboratory in a sealed plastic bag to prevent contamination, and then stored at –4°C until use. Samples were used for testing within their period of expiry.

Enrichment and isolation

For each of the chicken samples tested 5 g of the chicken sample was weighed and added to 50 ml of distilled water. It was then homogenised using a homogeniser 1 ml of this homogenate was then added to 10 ml of buffered peptone water. The samples were then incubated at 37°C for 16–24 hr. A 1 ml aliquot was removed and placed into test tube containing 10 ml of the Rappaport Vassiliadis (RV) enrichment broth (Rappaport et al., 1956; Vassiliadis et al., 1981a,b) and incubated at 43°C for 24 hr. Enriched broth was serially diluted upto 10^{-7} and 100 µl of each dilution (10^{-4} to 10^{-7}) was removed and plated onto Hektoen Enteric (HE) agar (Andrews et al., 1979). The plates were then incubated at 37°C for 24–48 hr. Selected colonies from the HE agar were purified by quadrant streaking on Luria-Bertani (LB) agar. The gram-staining was carried out on all the isolates after repeatedly purifying colonies on LB plate to observe the microscopic characteristics and to check for purity of *Salmonella isolates*.

FISH analysis

FISH analysis of the isolates from the chicken samples was carried out to identify *Salmonella* spp. using the VIT-Salmonella kit (Vermicon Identification Technology, Munich, Germany) according to the manufacturer's instruction. The slides were evaluated using 100X oil immersion lens of the epifluorescent microscope (NIKON eclipse 80i, Japan) fitted with the filters sets G-2E/C for (TRITC – tetramethylrhodamine isothiocyanate) and B-2E/C (for FITC – Fluorescein isothiocyanate).

Antimicrobial susceptibility testing

The sensitivity of the isolates to antibiotics was observed using the Kirby Bauer disc diffusion technique (Bauer et al., 1966). The isolates were grown on autoclaved Mueller Hinton broths (HiMedia, Mumbai, India) for 18 hr at 37°C. About 100–300 µl of the inoculum was spread plated onto petri plates of 120 mm in diameters containing autoclaved Mueller Hinton agar using sterile disposable L shaped spreaders. Only three antimicrobial discs were placed per plate using sterile forceps. The discs were placed at least 24 mm away from each other. The plates were incubated at 37°C for 18–48 hr until moderate growth was seen on the agar plates. The diameter of the zone of inhibition was then noted. The diameter zone inhibition

was then classified as being resistant, intermediate or sensitive based on standard zone sizes given by the antibiotic discs manufacturer (HiMedia, Mumbai, India).

RESULTS

Isolation of Salmonella from chicken meat samples

A total of 28 fresh chicken meat samples out of 60 found to be positive for *Salmonella that* is about 46.67% of the total samples. Samples obtained from the supermarkets tested negative for *Salmonella* while chicken samples obtained from the butcheries tested positive. About 54.55% of the chicken breast samples tested positive while 22.22% and 60% of the chicken thigh and chicken wing respectively tested positive (Table 1). A total of 66 *Salmonella* isolates were obtained through the culture dependant method, which includes non-selective preenrichment followed by selective enrichment and plating on selective and differential agar. All the isolates were found to be gram-negative small rods and have shown blue green colony with dark black centre that is the characteristic feature of hydrogen sulphide producing *Salmonella* spp. on HE agar.

Molecular identification of Salmonella isolates

FISH was carried out on all 66 *Salmonella* isolates. All 66 isolates were successfully hybridised by salmonella specific oligonucleotide probe confirming that isolates obtained through culture dependent method belonged to the Salmonella genus. Figure 1 shows the hybridisation of an isolate of *Salmonella* by fluorescently labelled oligonucleotide probe using VIT-Salmonella kit.

Antimicrobial susceptibility of Salmonella isolates

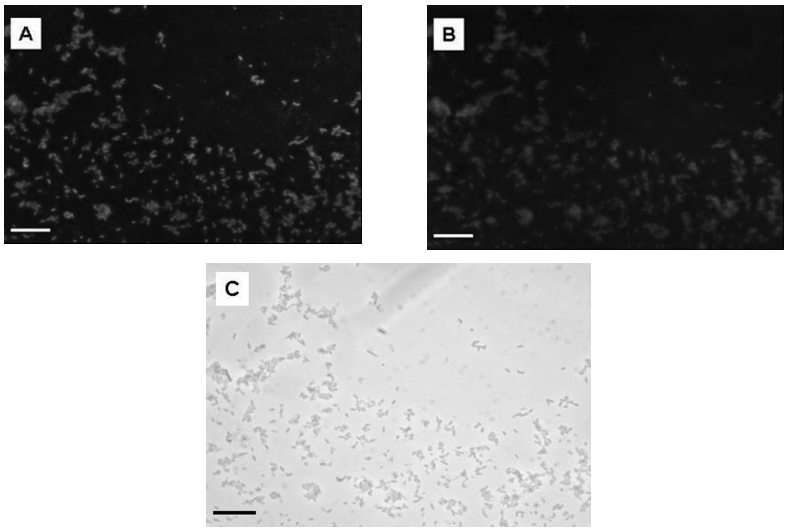
A total of 66 isolates of *Salmonella* from fresh chicken meat were tested for antibiotic sensitivity against ten commonly used antibiotics belonging to different groups. It was found that all isolates (100%) were resistant to cephalexin and rifampicin while about 90% and 88% of the isolates were resistant to ampicillin and tetracycline, respectively. Amikacin and ciprofloxacin were the most effective antibiotics with about 87% of the isolates exhibiting sensitivity to them. Ceftizoxime proved to be effective also with 78.79% of the isolates being sensitive. About 58% of the isolates were resistant to chloramphenicol while 36.36% and 21.21% of the isolates were resistant against carbenicillin and cefaperazone, respectively. Out of the 66 isolates analysed 60 were found to be resistant to four or more antibiotics, which is 90.10% of the total isolates. The activity of the 10 antimicrobial agents against 66 isolates of Salmonella is presented in Table 2.

Table 1 Isolation of *Salmonella* spp. from various chicken parts

Part of chicken (n)	Number positive for Salmonella	Proportion (%)	Identity using VIT-Salmonella kit*
Breast (22)	12	54.55	+
Thigh (18)	4	22.22	+
Wing (20)	12	60	+

*Positive hybridisation with salmonella specific fluorescently labelled oligonucleotide probe.

Figure 1 Whole-cell hybridisation of an isolate of *Salmonella* from chicken meat samples. For each panel, identical fields were viewed by epifluorescence microscopy and phase contrast microscopy (a) hybridisation with TRITC-labelled Salmonella probe, (b) hybridisation with FITC-labelled Salmonella probe, (c) Phase contrast image



Note: Bαρ = 10 μm and magnification: ×1000 applies to all photomicrographs.

DISCUSSION

Foodborne diseases caused by non-typhoidal *Salmonella* spp. represent an important public health problem worldwide (Aarestrup et al., 2007), therefore *Salmonella* has now become and important pathogen in the United Arab Emirates also. With *Salmonella* being found as the most common cause of food poisoning in Dubai, United Arab Emirates as reported by Dubai Department of Health and Medical Services there is an increased importance for the detection and characterisation of *Salmonella* spp. in the United Arab Emirates. To our knowledge, this is the first study to document data on the prevalence of *Salmonella* spp. from chicken meat samples in the United Arab Emirates. Due to the lack of insufficient documented information about *Salmonella* species in the gulf region an attempt was made to determine the dominance of *Salmonella* spp. in fresh chicken meat samples from various supermarkets and butcheries in the United Arab Emirates. The antibiotic susceptibility testing was carried out to determine their resistance pattern in UAE. Conventional preenrichment and enrichment culture techniques in combination with molecular identification procedures such as FISH provided a fast and accurate tool for the detection of *Salmonella*.

Fresh Chicken Samples from butcheries and supermarkets in the United Arab Emirates were tested for *Salmonella* spp. The absence of *Salmonella* spp. in retail poultry (those obtained from supermarkets) reflects the possibility that industry has managed to prevent effectively prevent spread of this microorganism during the marketing of meat. However, the high degree (100%) of *Salmonella* found in butcheries could be due to the unsanitary and unhygienic conditions of these butcheries. The improper handling of raw meat may have also caused this high

Table 2 Antimicrobial susceptibility pattern of *Salmonella* isolated from chicken meat samples by disk diffusion method

		Number of isolates (%) in susceptibility range		
Antimicrobial agents (symbol)	**Disc concentration (μg/disc)**	**Susceptible**	**Intermediate**	**Resistant**
Amikacin (Ak)	30	58(87.88)	4(6.06)	4(6.06)
Ceftizoxime (Ck)	30	52(78.79)	2(3.03)	12(18.18)
Cephalexin (Cp)	30	0(0)	0(0)	66(100)
Rifampicin (R)	5	0(0)	0(0)	66(100)
Cefoperazone (Cs)	75	16(24.24)	36(54.55)	14(21.21)
Carbenicillin (Cb)	100	28(42.42)	14(21.21)	24(36.36)
Ampicillin (A)	25	0(0)	6(9.09)	60(90.91)
Tetracycline (T)	10	0(0)	8(12.12)	58(87.88)
Chloramphenicol (C)	30	14(21.21)	14(21.21)	38(57.58)
Ciprofloxacin (Cf)	5	58(87.88)	6(9.09)	2(3.03)

level of contamination. The contamination of microorganisms in poultry products are reportedly derived from the poultry manure, poultry workers, equipment, poultry's environment which include faecal, soil and water. The slaughtering-plant operations generally amplify the level of bacterial contamination as was observed in this study. A study in India by Kamat et al. (2002) included different seafood products processed in six industries (three European Union Approved (EUA)) and three EU non-approved (EUN). The seafood products were analysed based on bacteriological analytical manual online for detection, enumeration and identification to species level of individual organisms (BAM, 2001). It was observed that 16.7% of cuttle fish, 28.5% of the shrimp and 40% of the squid EUN industries were positive to *Salmonella*. However, no positive samples of *Salmonella* spp. were found in the EUA plants. This was a good indicator that when Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Point (HACCP) were applied, their use resulted in a good product. Hence, implementation of practices such as GMP and HACCP may also help curb the prevalence of *Salmonella* spp. in chicken meat samples in United Arab Emirates.

Our findings of contamination rates with *Salmonella* were lower than those observed in the other countries, as 65.4% in the USA (Waltman et al., 1992), 57% in Portugal (Machado and Bernardo, 1990) but higher than 1.2% in Scotland (Brown et al., 1973). A study made in Turkey in between January 2000 and July 2001 and from July 2001 until December 2003 by Eyigor et al. (2005) revealed that *Salmonella* organisms were detected in 4.10% and 5.52% of chicken samples, respectively. When comparing our results to those of studies performed in Turkey, the data of the prevalence of *Salmonella* spp. from chicken in the UAE were lower than the results (69.77%) in Bursa district (Carli, 1990; Kucuker et al., 1993). Studies of meat and poultry products revealed *salmonellae* in Canada for the period 1983–1986 in 60.9% of 670 chicken samples (Lammerding et al., 1988). In a Spanish

study on 192 chicken livers and carcasses, 80% and 60% of samples, respectively were positive for *Salmonella* (Carraminana et al., 1997). In Venezuela, 41 out of 45 chicken carcasses studied yielded *Salmonella* (Rengel and Mendoza, 1984). However, when comparing our results to those of other authors, several factors must be taken into consideration, such as differences in origin, time period and age of the samples, sampling procedure, contamination level of animals, slaughterhouse sanitation, level of processing and cross contamination of the products, and differences in methodology applied to detect the pathogen.

FISH technique has become a routine molecular tool for analyses of the microbial community since it was first applied by DeLong et al. (1989). FISH was carried out for the 66 Salmonella isolated from fresh chicken meat samples. Salmonella was identified in 100% of the samples using the Vermicon Identification Technology (VIT) kit. This kit enabled a fast and unequivocal qualitative determination as to whether specific bacteria are present in the sample or not. The results were available within 3 hr. This test leads to a time-saving of approx. three days when compared with conventional methods. Thus, FISH proved to be a rapid and highly specific nucleic acid-based method for the whole-cell identification of bacteria.

In the current study the isolates were considerably less resistant to the third generation cephalosporins–cefaperazone and ceftizoxime with only 21.21% and 18.18% isolates showing resistance respectively. The two most effective antibiotics were found to be ciprofloxacin which is a member of the fluoroquinolone class of antibacterials and amikacin, an aminoglycoside antibiotic. To both of these antibiotics 87.87% of the isolates were sensitive. Ampicillin (β-lactam group), chloramphenicol (Phenicols), tetracylcine proved to be mostly ineffective while rifampicin (rifamycin group) and the first-generation cephalosporin, cephalexin were the least effective antibiotics with the isolates being 100% resistant to them. A study conducted by Rotimi et al. (2008) found that amikacin, cefotaxime, ceftriaxone, ciprofloxacin had excellent activities against all Kuwait and UAE isolates of Salmonella. These results are similar to those obtained in the current study. The resistance rates in Kuwait and UAE to ampicillin were 26.5% and 17.1%, cefotaxime/ceftriaxone 1.6% and 1.6%, ciprofloxacin 1.2% and 0.8%, chloramphenicol 5.6% and 5.7%, respectively. The resistance to Ampicillin (90.91%) and Chloramphenicol (54.54%) was of much higher degree. A total of 9.8% of the Kuwait isolates were multidrug resistant versus 4.1% of UAE isolates. In contrast out of the 66 isolates obtained from chicken meat samples in the UAE 96.67% of the isolates were resistant to three or more antibiotics. This sheds light on the high degree of antibiotic resistance that may be prevalent in the Gulf region. *Salmonella* strains resistant to antibiotics are commonly found worldwide, and this may be due to the comprehensive use of antibiotics included in feeds as growth promoters and due to the widespread use of antibiotics in chickens. The results of the antibiotic susceptibility testing showed a high degree of resistance for tetracycline (87.88%). Berchieri et al. (1983) verified 77% resistance to tetracycline of *Salmonella* in poultry feed and Antunes et al. (2003) found 36% of *Salmonella* strains resistant to tetracycline in broiler carcasses. Thus, the results obtained show a much higher degree of resistance to tetracycline than that observed by Berchieri et al. (1983) and Antunes et al. (2003). Bokanyi et al. (1990), Lee et al. (1993) and Nascimento et al. (1997) showed results, with 100% of sensitivity to chloramphenicol in broiler carcasses. However, chloramphenicol resistance was observed in 54.54% of the isolates in the current study. Lee et al. (1993)

and Oliveira et al. (2006) found 100% of resistance to ampicillin of *Salmonella* in broiler carcasses. The results obtained in the study showed that 90.91% isolates were resistant to ampicillin and 36.36% to carbenicillin. Antunes et al. (2003) found 19% and 3% of resistance carbenicillin and chloramphenicol, respectively. The low level of resistance to quinolones may be because they are relatively new antibiotics and are also more expensive than the tetracycline, ampicillin and chloramphenicol. We also observed two low molecular weight plasmids (700 and 1000 bp) in the most of the *Salmonella* isolates obtained in this study (data not shown). The presence of these plasmids could explain the high degree of antibiotic resistance observed in the isolates (Carattoli, 2003). In a study in Benin City, Edo State, Nigeria (Suh and Odeh, 2008) 32 (17.5%) of the isolates had plasmids of varying sizes ranging from 2.5 to 5.0 kb while 151 (82.5%) appear to have no plasmids. *Salmonella enterica* serovar Enteritidis frequently contains plasmids. The best understood is the serovar-specific plasmid which encodes for virulence genes, for example, *spv, pef* or *rck*. Besides this plasmid, wild-type *Salmonella* serovar Enteritidis strains occasionally contain additional plasmids, frequently of low molecular weight. They have been suspected to influence resistance to antibiotics. In the present research *Salmonella* isolates resistant to eight out of the ten antibiotics were found. Hence, stringent antimicrobial prescription policies in both human and veterinary medicine, as well as improvements in public health and infection control policies, are required to reduce the biological and economic burden of *Salmonella* infections in UAE. The presence of low molecular weight plasmids in the study along coupled with the high antibiotic resistance observed indicates that these plasmids may be a vehicle for the rapid transfer of antibiotic resistance markers of *Salmonella* species. Majority of *Salmonella* species are potentially pathogens therefore pose public health risk. The transfer of antibiotic resistant genes among the species is an increased risk hence, further research through conjugative studies is required to determine the role of plasmids in the transfer of antibiotic resistance. There is also a need for determining the prevalence of *Salmonella* in other food items such as fruits, vegetables, eggs and other meat products like beef and pork in order to get a comprehensive overview of the pervasiveness of *Salmonella* in the United Arab Emirates.

CONCLUSIONS

This study shows the prevalence of *Salmonella* in fresh chicken meat samples in Dubai, United Arab Emirates. *Salmonella* was found to be dominant in samples obtained from butcheries while it was found be absent in samples from supermarkets. In total 66 pure isolates of *Salmonella* were obtained after following systematic culture dependant procedure of preenrichment, selective enrichment, isolation on selective agars and purification. Molecular characterisation by FISH based kit for *Salmonella* detection further confirmed the identity of all *Salmonella* isolates. In order to detect for the presence of drug resistant and susceptible isolates, all the isolates of *Salmonella* were subjected to antimicrobial susceptibility testing using disc diffusion assay. All the isolates that is, 100% showed resistance to cephalexin and rifampicin. A high degree of resistance was also observed for ampicillin and tetracycline while 87.88% of the isolates were sensitive to ciprofloxacin and amikacin. The prevalence of high degree of multiple drug resistant Salmonella spp. in chicken meat products in Dubai indicates that drug resistance of *Salmonella* is becoming a crucial problem in this part of the world and may pose a great health risk.

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