Studies on Retail Chicken Meats With A Special Reference to Antibiotic Resistant Bacteria

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Abstract
This study was conducted on retail chicken meats to determine the prevalence of antibiotic resistant bacteria.The prevalence of antibiotic resistant bacteria in retail chicken meat was determined in hail region, Saudi Arabia. A total of 50 samples were collected and examined for the presence of antibiotic resistant bacteria. *E. coli* was the most prevalent isolates (48%) followed by *S. aureus* (24%) and *Salmonella* (20%). Some of them were multi-drug resistant (MDR). Resistance of *S. aureus, E. coli* and *Salmonella* spp. to tetracycline was most frequent at 25%, 79.16% and 60%, respectively. These findings suggest that the abundant presence of multi-drug resistant bacteria in the chicken meat may have a profound effect on future treatment options for a wide range of infections caused by bacteria.
Key words: chicken meats, Antimicrobial resistant; MDR; *E. coli; Salmonella and S. aureus.*

Introduction

Chicken meat can make numerous positive commitments to the eating routine of those on low livelihoods. Despite not all meats are seen Likewise healthy, chicken meat is cheap now and again more moderate than different meats. It can be advanced with some key supplements around the world as it is of a consistently high quality, is low in saturated fats.

There has been expanding worry on the rise of multidrug-resistant foodborne pathogens from food, including poultry ***(Kilonzo-Nthenge et al., 2013).*** contamination of food with antibiotic-resistant foodborne pathogens continues to be a major risk to public health and potentially compromises the treatment of severe bacterial infections ***(Van et al., 2007).*** The resistance of bacteria to antimicrobials will continue to threaten the therapeutic use of antibiotics in clinical medicine if massive use of antibiotics is not restricted ***(McGowan Jr, 2001)***. Antimicrobials are used to improve animal growth and disease control ***(Tollefson and Miller, 2000)***. the used antibiotics in treatment of animal and human are mostly the same consequently expanding the danger of development and spread of resistant bacteria in both animals and humans ***(W.H.O., 2007).*** Tetracyclines, aminoglycosides, βlactams, lincosamides, macrolides, and sulfonamides were broadly utilized in poultry and animal farms ***(Lee et al., 2001).***

Antibiotics are used to control the susceptible bacterial infection, at a therapeutic dose, while strains with unusual traits can be developed and can multiply and exhibit resistance to antibiotics (Apata, 2012). Some of the antimicrobial resistant genes detected in isolated bactreia from food also identified in humans due to consumption of food containing resistant bacteria or through contact and this accentuate the indirect transfer by food handling and/or consumption (Marshall and Levy, 2011). So the purpose of this study was to determine the prevalence of drug-resistant bacteria in chicken meat.

Materials and methods

Samples collection and preparation:

Fifty whole chicken carcasses samples were purchased from different groceries in Hail region, Saudi Arabia. Twenty five grams of each sample were homogenized in 225 ml of buffered peptone water (BPW), under aseptic conditions for 2 min. by using a sterile homogenizer.

Isolation and Identification of bacteria:

Isolation of the foodborne pathogens *E. coli , Salmonella and S. aureus and K. pneumoniae* were carried out according to ***(BAM et al., 1998).*** Identification of bacterial strains was performed by using conventional methods including catalase, coagulase and mannitol fermentation tests, and Gram-staining. Further the identification was confirmed by MALDI-TOF and Microscan, according to the manufacturer's guidelines of ***(van Veen et al., 2010)***. A single colony of a (sub) culture was directly deposited in duplicate on a MALDI-TOF-MS plate (Bruker Daltonik GmbH, Germany) and the results were recorded

Susceptibility testing of isolated bacteria:

Isolates were tested for susceptibility to antimicrobials by Microscan method [susceptibility based on minimum inhibitory concentration (MIC)].The isolates were also tested for their antimicrobial susceptibilities by the disc diffusion technique using the following antibiotic discs for Gram positives: amoxicillin (30 µg), ampicillin (10 µg), cefepime (30 µg), cefotaxime (30 µg), cefuroxime (30 µg), cephalexin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), methicillin (5 µg), oxacillin (1 µg), rifampicin (5 µg), roxithromycin (15 µg), streptomycin (10 µg), trimethoprim-sulfamethoxazole (5 µg) and vancomycin (30 µg). For Gram negatives the antimicrobials tested will be as follows, ampicillin (10 µg), cotrimoxazole (25 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), ceftriaxone (30 µg), nalidixic acid (10 µg), meropenem (10 µg), tigecycline (15 µg), colistin (10 µg) and polymixin (300 units). MIC values were determined following (Watts et al., 2008) interpretative standards.

Results and Discussion

The standard treatment of infections can be affected due to resistant bacteria resulting in bad prognosis and a greater risk of death. Antimicrobial-resistant bacteria in animals are a growing concern because of their potential for transmission to humans as foodborne pathogens ***(Welton et al., 1998; Witte, 1998)***. Antibiotic resistant bacteria and resistance genes can be evolved and transferred to people through the consumption of meat or even through direct contact with food animals which in turn threaten the effective prevention and treatment of bacterial infections ***(Darwish et al., 2013).*** The emergence of antibiotic resistance can increase the difficulties of human treatment and this might be increased by the indiscriminate use of antibiotics in poultry and animal farm., so it is better to use some classes of antibiotics for animal diseases treatment and other classes for human treatment ***(Darwish et al., 2013)***

The incidences of bacteria found in chicken meat are shown in figure 1. *E. coli* was the most frequent bacterial contaminant by 48% followed *S. aureus* (24%), *Salmonella* (20%) and *K. pneumoniae* (16%) . Although most strains of *E. coli* are not regarded as pathogens, they can be opportunistic pathogens that cause infections in immunocompromised hosts and there are also pathogenic strains of *E. coli* that when ingested, causes gastrointestinal illness in healthy humans ***(BAM et al., 1998)***. Drug-resistant strains of *E. coli* isolated from humans are originated from meat and meat products; these strains are highly resistant to fluoroquinolones and can spread into the community from food chains ***(Collignon, 2009).***

Salmonella is an important cause of food-borne illness and the development of resistant strains is associated with the extra use of antibiotics in animals; and can be transmitted to humans ***(White et al., 2001).*** Multidrug resistance was detected in 92·8% of the isolated *Salmonella* Which confirms the contamination of chicken meat with multidrug-resistant strains representing a danger to public health ***(Abd-Elghany et al., 2015)***.

S. aureus is a leading cause of food poisoning and many infections to human such as pneumonia and postoperative wound infections ***(de Boer et al., 2009; Horan et al., 1988).*** The presence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in raw meat may constitute a health hazard to consumers ***(KITAI et al., 2005).*** Recently, methicillin-resistant *Staphylococcus aureus* (MRSA) strains could be isolated from several food-producing animals ***(de Boer et al., 2009; KITAI et al., 2005; Lee, 2003).*** Further studies are expected to explain the transmission courses of MRSA in connection to meat and other foods and to give the abilities to ban the spread of MRSA. The emergence of MRSA in human infection significantly related to the high prevalence of MRSA in meat which consider a greater health hazard for consumers ***(de Boer et al., 2009)***. *K. pneumoniae* is responsible for infections of immunocompromised patients as it is an opportunistic pathogens associated with 2%–5% of nosocomial infections especially the urinary and respiratory tracts ***(Podschun and Ullmann, 1998).*** In the study of ***(Calbo et al., 2011)*** who reported the transmission of ESBL- *K. pneumoniae* through the food where food can be a transmission vector for multidrug-resistant strains in the hospital setting, and they should consider extending their surveillance to kitchen facilities and foodstuff. Another study reported *K. pneumoniae* as an enteroinvasive food-borne pathogen transmitted from a hamburger ***(Sabota et al., 1998).***

Contamination of chicken meat with antibiotic resistant bacteria was identified and represented in table 1. The level of resistance was high and many bacteria were resistant to more than one antibiotic. Results indicate that isolated *E. coli* was resistant to amoxicillin clavulanate, ampicillin, gentamicin, tetracycline, moxifolxacin and trimethoprim/sulfamethoxazole while *Salmonella* spp. were resistant to amoxicillin clavulanate, ampicillin, tetracycline, moxifolxacin and trimethoprim/sulfamethoxazole. *S. aureus* isolates were resistant to ciprofloxacin, tetracycline, moxifolxacin, cefoxitin, amikacin, trimethoprim /sulfamethoxazole. The antimicrobial susceptibility pattern of the *K. pneumoniae* showed a resistance to amoxicillin Clavulanate, ampicillin, tetracycline, vancomycin, cefoxitin and amikacin.

A bacterial resistance to multiple antimicrobials is adding to the problem of meat contamination from animal hides, feces, environments (Graham et al., 2009). The highest MDR was observed for *E. coli (91.6%)* followed by *S. aureus* (75%). Many studies in the Saudi Arabia reported the occurrence of antimicrobial resistant bacteria isolated from chicken mean ***(Altalhi et al., 2010; Greeson et al., 2013)***

Although at least some of these bacteria may not be pathogenic strains themselves, they are a cause of concern because they can extend antibiotic resistance to other bacteria **(Greeson et al., 2013).** Antibiotic resistant, but not susceptible from human infections in the United States were found to be indistinguishable from those in chickens suggesting a directional transmission of resistant bacteria from a food source ***(Weese et al., 2010).*** Many studies reported the emergence of many antimicrobial-resistant E. coli strains which were responsible for infections of the urinary tract and bloodstream in humans and originated from contaminated retail meat ***(Carlet et al., 2012).***

The obtained results reported the presence of antibiotic resistant bacteria isolated from chicken meat which may have a serious consequences for public health and can affect consumers. The occurrence of resistant bacteria could be attributed to the indiscriminate use of antibiotics in poultry farms. Data are lacking link between the uses of antibiotics in animals and the development of multiple drug resistant bacteria so the scientific community has been forced to follow and research for the reservoir of resistant microorganisms.

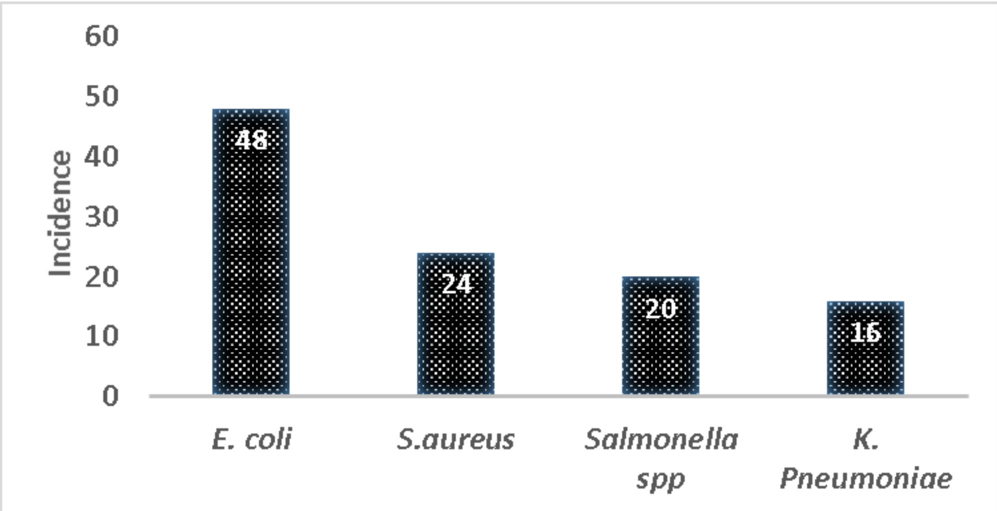

Figure 1. *Incidence of some bacteria in examined chicken meat samples (n=50).*
E. coli = Escherichia coli
S. aureus = Staphylococcus aureus
K. pneumoniae = Klebsiella pneumonia

Table 1. *Prevalence of drug-resistant bacteria isolated from raw chicken meat*

Antibiotic	*S. aureus* n= 12	*K. pneumoniae* n= 8	*E. coli* n= 24	*Salmonella* spp n= 10.
MDR*	9 (75%)	2 (25%)	22 (91.6%)	5 (50%)
Amoxicillin clavulanate	0(0)	4 (50%)	1 (4.16%)	1 (10%)
Ampicillin	0(0)	1 (12.5%)	15 (62.5%)	2 (20%)
Ciprofloxacin	1 (8.3%)	0(0)	0(0)	0(0)
Gentamicin	0(0)	0(0)	3 (12.5%)	0(0)
Tetracycline	3 (25%)	6 (75%)	19 (79.16%)	6 (60%)
Vancomycin	0(0)	1 (12.5%)	0(0)	0(0)
Moxifolxacin	1 (8.3%)	0(0)	6 (25%)	30 (30%)
Cefoxitin	1 (8.3%)	2 (25%)	0(0)	0(0)
Amikacin	1 (8.3%)	1 (12.5%)	0(0)	0(0)
Trimethoprim/sulfamethoxazole	1 (8.3%)	0(0)	2 (8.3%)	4 (40%)

**MDR Multi-drug resistant*

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دراسات على لحوم الدواجن خاصة بالبكتريا المقاومة للمضادات الحيوية

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أجريت هذه الدراسة على لحوم الدواجن لمعرفة مدى انتشار مقاومة البكتيريا للمضادات الحيوية. تم تجميع ٥٠ عينة من لحوم الدجاج الصالحة للإستهلاك الآدمى عشوائياً من أماكن بيع مختلفة في مدينة حائل بالملكة العربية السعودية. تم عزل ميكروبات الإشريكية القولونية والمكور العنقودي الذهبي والسالمونيلا من لحوم الدجاج بنسب ٤٨٪، ٢٤٪ و٢٠٪ على التوالي. كانت مقاومة العزلات البكتيرية للتتراسيكلين هو الاكثر شيوعا وبنسب ٢٥٪، ٧٩,١٦٪ و٦٠٪ في الإشريكية القولونية والمكور العنقودي الذهبي والسالمونيلا على التوالي. تشير النتائج إلى وجود وفرة من البكتيريا ذات المقاومة المتعددة للمضادات الحيوية المقاومة في لحوم الدجاج والذي قد يكون له تأثير كبير على خيارات العلاج المستقبلية للعديد من الإصابات البكتيرية.