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Incidence of bacterial contamination in wastes of private poultry life markets slaughter premises in Sana'a city. I: Isolation, Identification and antibiogram of isolates.

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SUMMARY

This study was carried out to investigate the bacterial contamination and their drug sensitivity pattern in private poultry life markets and slaughter premises (shops) wastes in Sana'a city. Examination of collected 146 samples including intestinal contents (63), water from washing tanks (17), feathers (19), Spleen (25) and long bones (22) revealed 35,11,5,1 an 0 isolates in a rate of 55.5, 64.7, 26.3, 4 and 0%; respectively. The obtained bacterial isolates were subjected to colonial morphological, Gram stain reaction and biochemical identification. Bacteriological examination of samples revealed that 52 isolates were recovered in a total rate of 35.6%. These isolates were including 27 (51.9%) E. coli; 10 Citrobacter Freundii (19.3%); 3 (5.7%) Klebsiella Pneumoniae (K. Pneumoniae); 1(1.9%) Salmonella Enteritidis (S. Enteritidis); 3 (3.8%) Campylobacter jejuni (C. jejuni); 1 (1.9%) Pseudomonas aeruginosa (Ps. aeruginosa); 5 Staphylococcus coagulase negative (9.6%); and 3 Streptococcus β haemolytic (5.7%). Results of sensitivity of bacterial isolates against used 16 antibiotics. It was observed that no single antimicrobial drug was 100% effective against different isolates. It is clear that most isolates were resistant to Tetracycline, Oxytetracycline, and Erythromycin. We recommend the strict application of sanitary measures in life poultry markets to minimize bacterial contamination and spread of pathogens; especially to human. The obtained isolates will be subjected to pathogencity test for 1-day-old chicks.

INTRODUCTION

The Yemeni poultry sector represents one of the largest livestock sector investments in the country, with an estimated investment of over 2 billion US $. The poultry marketing

chain can be an important infrastructure in promoting or breaking the infectious poultry diseases. The marketing of poultry products in Yemen is governed by the consumer habit of selecting live poultry at the market and halal-slaughtered at the time of purchase. Live bird markets and its offal's play an important role in transmission of poultry pathogens as live birds enter poultry processing plant carrying large numbers of microorganisms on the feathers, skin, and in the alimentary tract (Mead, 1989). During the different processing stages, a large numbers of these microorganisms are removed, however further contamination can also occur from sources such as the environment, equipment surfaces and manual handling (Bryan, 1980 and Mead, 1989). Live –bird markets are distributed in the inner cities allover the country in which there facilities are rarely depopulated, cleaned, and disinfected. These markets act as ideal situation for sources, transmission and propagation of poultry diseases as well as public health hazards. On the other hand Antibiotic use whether for therapy or Key words: Live bird markets, bacterial contamination, Antibiogram Author prevention of bacterial diseases, or as performance enhancers will result in antibiotic resistant micro-organisms, not only among pathogens but also among bacteria of the endogenous microflora of animals. The extent to which antibiotic use in animals will contribute to the antibiotic resistance in humans is still under much debate. In addition to the veterinary use of antibiotics, the use of these agents as (AGP) greatly influences the prevalence of resistance in animal bacteria and a poses risk factor for the emergence of antibiotic resistance in human pathogens (WHO,1997; Aarestrup,1999, Van den Bogaard and Stobberingh, 1999. and Tollefson and Miller, 2000).

From the above mentioned, this work was initiated to investigate the following:

a- Incidence of various bacterial pathogens among poultry waste products in some primitive slaughter shops with cultural, morphological and biochemical identification of bacterial isolates.

b- Antibiogram of the obtained bacterial isolates.

MATERIAL AND METHODS

1- Samples:

A total of 146 samples including intestinal contents (63), water from washing tanks (17), feathers (19), Spleen (25) and long bones (22) were obtained from different slaughter places in local live poultry markets in Sana'a city.

2- Media for isolation and identification:

I. The following media were used for isolation of possible bacterial content of examined samples as follows:

a. Enterobacteriaceae: Xylose lysine deoxycholate (XLD) agar and MacConkey,s

agar medium (Oxoid). Nutrient broth, Selenite-F-broth and Mueller Hinton broth (Wilson and Miles, 1975).

b. Genera Staph and Streptococcus: Nutrient agar medium, Blood agar medium and brain heart infusion agar (Difco). Brain heart infusion broth and Mueller Hinton broth (Wilson and Miles, 1975).

c. Campylobacter: Skirrow's Medium and Butzler and Skirrow's (1979) medium from Difco were used.

d. Pseudomonas aeruginosa: Nutrient agar medium (Oxoid).

II. Biochemical reaction:

a. Enterobacteriaceae: API 20E and API 20NE strips commercial kit systems from Difco were used.

b. Staph and Streptococcus: API 20 Staph and Strept commercial kit system Difco were used.

c. Campylobacter: The used media and tests were used according to Collee et al. (1996).

d. Pseudomonas aeruginosa: API 20E kit, bio Merieux was used (Collee et al., 1996).

III. Media for Antibiogram (Antibiotic Sensitivity test):

Muller Hinton agar media was use for Antibiotic sensitivity testing of the obtained 52 bacterial isolates using the disk diffusion technique (Bauer et al, 1966).

4- Antibiotic Sensitivity disks:

Sixteen types of antibiotic discs produced by Oxoid were used. The degree of sensitivity was interpretated according to (Anon 1982).

5- Bacteriological examination:

Identification of the obtained isolates was done on the basis of cultural morphological, Gram stain and biochemical reaction according to Quinn et al. (1994) and collee et al. (1996).

Collected samples were prepared for Bacteriological examination and cultured in fluid media including, nutrient broth incubated at 37°C for 24 hours and Selenite-F-broth incubated at 43°C for18 hours for Enterobacteriaceae.

Subcultures from broth media were cultured onto solid agar media including MacConkey and Xylose lysine deoxycholate agar media for Enterobacteriaceae, Nutrient, Blood and brain heart infusion agar media for Gram- positive bacteria as well as Skirrow's , Butzler and Skirrow's media for Campylobacter and Nutrient agar medium for Pseudomonas aeruginosa. All culture media were incubated for the recommended temperature, time and precaution then examined for bacterial growth.

RESULTS

1. Isolation of bacterial agents:

From 146 samples including intestinal contents (63), water from washing tanks (17), feathers (19), Spleen (25) and long bones (22) were obtained from different local live ansd slaughter poultry market shops located in Sana'a city (Table 1).

2. Identification of Bacterial isolates (Table 2): Colonial morphology, Gram staining reaction, organism morphology and biochemical properties of the obtained bacterial growth were recorded as follows:

1. Bacterial growth on MacConkey and XLD media was suspected to be Enterobacteriaceae members colonies measured 2-3 mm in diameter. They were Gram-negative, non spore forming bacilli. Forty one Enterobacteriaceae isolates were biochemically classified into 27 E. coli, 10 C. freundii, 3 K. pneumoniae and 1 S. Enteritidis.

2. Bacterial growth in form of small dew drop like colonies on Nutrient and Brain heart infusion agar were supcultured on blood agar, 3 isolates were small, and usually grayish in color after 24-48 hours of incubation at 37°C and could appear from mucoid to rough, causing complete haemolysis and greenish discoloration, were suspected to be Streptococcus β haemolytic from intestinal samples. The organisms were Gram-positive oval coccid, in singly or in pairs or short chains.

3. Colonies onto nutrient, blood and brain heart infusion agar media of 5 isolates obtained from intestine were smooth, low convex, glistening gray or white in color and organism proved to be Gram-positive arranged in a grape like clusters were suspected to be Staphylococcus. The obtained isolates were further identified as Staphylococcus coagulase negative

4. Colonies onto Skirrow's medium containing; Vancomycin, polymeric and Trimethoprim in presence of 5% oxygen and 10% CO2 of 2 isolates were defuse droplet-like colonies after 24-48 hours at 42 °C. The organisms were small Gram-negative rods comma or S- shaped and suspected to be Campylobacter and biochemically were identified to be C. Jejuni.

5. Colonies onto nutrient agar leading to greenish coloration of the medium was suspected to be Ps. aeruginosa, Gram-negative bacilli, non capsulated and non sporing.

3. Sensitivity and susceptibility of bacterial isolates against used 16 antibiotics (Table 3). It was observed that no single antimicrobial drug was 100% effective against different isolates. It is clear that most isolates were resistant to Tetracycline, Oxytetracycline, and Erythromycin.

Table (1): Incidence of bacterial isolation from examined slaughter waste samples.

NO	Samples		No of positive isolates	Isolation %
	Types	Number		
1	Intestinal content	63	35	55.5
2	Water washing tanks	17	11	64.7
3	Feathers	19	5	26.3
4	Spleen	25	1	4
5	Long bone (bone marrow)	22	0	0
Total		146	52	35.6

DISCUSSION

Marketing and on-site slaughtering takes place in thousands of small poultry shops in cities, towns and villages which play an important role in transmission of different bacterial pathogens. On other hand, since the discovery and development of the first antibiotics prior to the Second World War, these drugs have played an important role in curing disease in humans and animals. So, the antimicrobial drugs are extensively used in veterinary practice for the treatment and control of many bacterial, fungal and protozoal diseases of animals and poultry. This fact associated with the misuse of such therapeutic agents (incomplete antibiotic courses, insufficient doses and the blind use of non- specific agents) in the developing countries resulted in the development of multiple antibiotic resistant strains (e.g. Pseudomonas, Salmonella, E. coli and Staphylococci ...etc (Levy, 1992).

Table (2): Typing and Incidence of identified bacterial isolates from slaughter waste samples according to their biochemical properties.

Bacterial isolates		Samples (type &No)					Total
Type	No & %	Intestine (63)	Wash water (17)	Feathers (19)	Spleen (25)	Bone marrow (22)	146
E. coli	No	18	6	2	1	0	27
	%	28.6	35.3	10.5	4	0	18.5
C. Freundii	No	7	2	1	0	0	10
	%	11.1	11.7	5.3	0	0	6.8
K. pneumoiae	No	2	1	0	0	0	3
	%	3.2	5.8	0	0	0	2.1
S. Enteritidis	No	1	0	0	0	0	1
	%	1.6	0	0	0	0	0.7
Strept.	No	1	1	1	0	0	3
	%	1.6	5.8	5.3	0	0	2.1
Staph.	No	4	0	1	0	0	5
	%	6.4	0	5.3	0	0	3.4
C. Jejuni	No	2	0	0	0	0	2
	%	3.2	0	0	0	0	1.4
P. aeruginosa	No	0	1	0	0	0	1
	%	0	5.8	0	0	0	0.7
Total	No	35	11	5	1	0	52
	%	55.5	64.7	26.3	4	0	35.6

Antimicrobial therapy, when indicated is often initiated before the results of susceptibility testing are available. Thus a general knowledge of the expected susceptibility of bacterial species causing infections in a given geographic region is a prerequisite to initiate treatment with the most appropriate antimicrobial drug.

On screening the prevalence of bacterial contents of the collected 146 samples (63 intestinal content, 17 washing water, 19 feather samples , 25 spleen and 22 long bones) from live and slaughter poultry markets (shops) located in Sana'a capital city. The identification of bacterial isolates was carried out on the basis of morphological and

microscopical examination, cultural and biochemical reaction .The results of colonial morphology and microscopic features of 41 Enterobacteriaceae isolates were in accordance to those described by Carter and Cole (1990), Quinn et al. (1994), Eisa (1995) and Colee et al. (1996) where E. coli, C. freundii, K. pneumoniae and S. Enteritidis were isolated. The results obtained of biochemical behavior of each member of Enterobacteriaceae are similar to those described by Quinn et al. (1994), Collins et al. (1995) and Colee et al.(1996). The results with bacterial growth and microscopic examination of 8 Gram + ve isolates proved to be 5 Staphylococci and 3 Streptococci, this come in accordance to those described by Colee et al. (1996). The results of biochemical reaction of Gram + ve isolates were similar to those of Quinn et al. (1994).

The results of bacterial growth on to Skirrow's medium suspected to be C. jejuni comes in accordance to those described by Colee et al. (1996). On the other hand colonies growth on nutrient agar medium leading to greenish coloration of the medium is suspected to be Ps. aeruginosa. The results of biochemical reactions of C. jejuni and Ps. aeruginosa isolates were similar to those described by Koneman et al.(1994) and Colee et al.(1996).

Concerning the bacterial isolates identifications shown in table (2). It was clear that most isolates were E. coli (27/52) with a percentage of 18.5, which seem to be in agreement with those found by Shooter et al. (1974) who succeeded to isolate 349 strains of E. coli from chicken rectal swab, feather and washing water, Cox and Blankenship (1975) who estimated E. coli in chiller water in a rate of 96.4% , Fliss et al. (1991) who found E. coli and coliforms from poultry carcasses and attributed that to the perforation of the digestive tract during slaughtering processing. Moreover, Turtura (1991) identified 65 E. coli 17.6% isolates out of 369 bacterial strains from rinsing water, while E. coli could be detected in 41.1% of poultry carcasses by Cenci et al .(1992), Eisa (1995), dipping water Abu- Ruwaida et al (1994).

Ten isolates of C. freundii could be isolated with total percentage of 6.8% out of total examined samples (Table 2) .Cenci et al. (1992) could isolate Citrobacter from 15.7% of examined poultry carcasses, Eisa (1995) Identified C. freundii out of Coliforms obtained from chicken carcasses in private shops. Moreover; Russell et al (1993) and Abu- Ruwaida et al. (1994) isolated Coliform bacteria including Citrobacter from offal's and dipping water samples.

Three K. Pneumoniae isolates succeeded to be identified with total percentage of (5.7%) out of total examined samples. Cox and Blankenship (1975) found that Enterobacteriaceae in chiller water and on poultry carcasses were mainly of genus Escherichian, while Klebsiella was less

frequently detected. Cenci et al. (1992) isolated Klebsiella at a rate of (5.6) of total (427) poultry carcasses samples examined. Eisa (1995) found that the total Coliform count including Klebsiella at Governmental abattoirs was far less than those in private shops.

S. enterica represents a leading cause of food borne infections worldwide and includes more than 2500 different serovars (Popoff, 2001). Only One isolate of S. Enteritidis was obtained from intestine with a total percentage of (1.9%). Our results agree with those of Cox and Blankenship (1975) where Salmonella were less frequently detected. Biro and Nagy (1989)

Table (3): Antibiogram (In Vitro sensitivity) test of the obtained 52 bacterial isolates.

Antibiotic	Sensitivity of bacterial isolates							
	E. coli	C. Freundii	K Pneumoniae	S Enteritidis	Strept	Staph	C. Jejuni	Ps. aeruginosa
Amoxicillin AMX 25 µg	++ (16) - (11)	+++ (1) ++ (3) - (6)	++ (1) - (2)	+	++ (1) + (1) - (1)	++ (3) + (1) - (1)	ND	–
Nalidixic acid NAL 30µg	++ (9) - (18)	+ (3) - (7)	+ (2) - (1)	-	+ (1) - (2)	ND	++	–
Colistine 10µg CL	++ (8) - (19)	++ (2) - (8)	+ (1) - (2)	-	–	–	+	++
Doxycycline D30µg	++ (6) - (21)	++ (1) - (9)	- (3)	–	++ (1) + (1) - (1)	+ (2) - (3)	–	–
Ampicillin AMP,10µg	+ (7) - (20)	++ (3) - (7)	+++ (1) - (2)	-	++ (2) - (1)	++ (3) + (2)	+	–
Chlormphenicol CHL, 30µg	+++ (5) ++ (12) - (10)	+++ (3) ++ (2) - (5)	++ (1) + (1) - (1)	++	+ (2) - (1)	++ (4) - (1)	++	–
Neomycin N, 10µg	++ (11) + (7) - (9)	+++ (1) ++ (4) - (5)	++ (2) + (1)	+	(2)+ - (1)	+++ (1) ++ (3) - (1)	–	–
Streptomycin S 10µg	+++ (7) + (12) - (8)	++ (4) - (6)	++ (3)	+	+ (2) - (1)	+ (4) + - (1)	+	++
Tetracycline TET, 30µg	+ (4) - (23)	+ (2) - (8)	- (3)	-	+	- (4) + (1)	-	-
Oxytetracycline OXY, 30µg	+ (6) - (21)	++ (1) - (9)	- (3)	-	-	- (5)	-	-
Erythromycin ERY, 15µg[	+ (8) - (19)	++ (3) - (7)	+ (1) + (2)	+	++ (2) + (1)	+++ (3) ++ (2)	+	-
Gentamicin GM,120µg	+++ (9) + (11) - (7)	++ (4) - (6)	++ (2) + (1)	++	+ (1) - (2)	++ (2) + (2) - (1)	++	+
Flumequine UB, 30µg	+++ (10) + (7) - (10)	+++ (1) ++ (5) - (4)	++ (2) + (1)	-	ND	ND	-	+
NorfLoxacin NF,5µg	+++ (17) ++ (8) - (2)	+++ (7) ++ (2) - (1)	+++ (2) ++ (1)	++	+++ (1) ++ (2)	+++(3) ++(1) +(1)	-	+++
Ciprofloxacin Cip,5µg	+++ (12) ++ (10) - (5)	+++ (6) ++ (3) - (1)	+++ (1) ++ (2)	++	+++ (2 ++ (1)	+++(3) ++(2)	-	++
Furazolidin	++ (6) + (8) - (13)	+++ (1) + (6) - (4)	++ (1) + (1) - (1)	+++	++ (2) + (1)	+++ (3) ++ (2)	-	-

+++: Highly sensitive. ++: Moderately sensitive. +: Slightly sensitive.
- : Resistant. ND: Not done.

and Carovergara and Pascual (1989) isolated S. Enteritidis from different abattoirs. Ishioka et al (1997) isolated Salmonellae from broiler processing in Japan. Moreover; Capita et al (2007) succeeded to isolate S. Enteritidis from chicken carcasses in slaughterhouses in Spain. While, Turtura (1991) could not isolate Salmonella from different slaughterhouse.

Five isolates of Staphylococcus coagulase negative were obtained from examined samples with total percentage of (9.6%) out of total examined samples (Table 2), Panda (1971) and Oliver et al (1996) isolated the organism from skin surface, while Anand et al (1989) recorded heavy contamination of Staphylococci in washing water. Moreover; Abu-Ruwida et al (1994) reported that carcasses were heavily contaminated with Staphylococci. Vural et al (2006) isolated Staphylococci from chicken carcasses and their products.

Three β- haemolytic Streptococci strains were obtained from intestine, washing water and feathers respectively with total percentage of (5.7%) out of total examined samples. Similar results were reported from fresh offals (Russell et al., 1993) and skin (Geornaras et al., 1996).

Two isolates of C. jejuni were obtained from intestinal samples with total percentage of (3.8%) out of total examined samples (Table 2). Hoop and Ehrsam (1987) isolated C. jejuni from skin and intestinal poultry samples, Dias et al (1990) found C. jejuni from non- industrialized poultry plants. Audisio et al. (1999) and Denis et al. (1999) stated that poultry has been shown to be the primary source of Campylobacter spp. As a food borne pathogen, Campylobacter is transmitted to human via contaminated food and water Allos, (2001). Particularly, the chicken is a natural host of C. jejuni and serves as a major reservoir for this pathogenic organism (Lee and Newell, 2006). Contamination of chicken carcasses by Campylobacter often occurs during the slaughtering process and consumption of chicken meat is a significant source of human Campylobacter infections (Humphrey et al., 2007). Thus control of Campylobacter in poultry should yield a positive impact on improving food safety.

Only 1 Ps. aeruginosa isolate was recovered from washing water tanks with total percentage of (1.9%) out of total examined samples (Table 2). Panda (1971) isolated Pseudomonas from skin surface of dressed birds . Ps. aeruginosa is an important opportunistic pathogen that causes serious infections in both humans and animals (Gales et al., 2001) and Harjai et al., 2005). It is well known, that Pseudomonas spp. including P. aeruginosa has natural resistance to many antimicrobials and antibiotics (Gallert et al., 2005 and Giamarellos-Bourboulis, 2008).

Negative results of bacterial isolation from bone marrow in the present study may

be attributed to sound health of examined birds.

The utilization of antimicrobial drugs has played an important role in animal husbandry, since they are used in prophylaxis, treatment and growth promotion. However, the extensive use of those in human and animals has led to an increase in bacterial multidrug resistant among several bacterial strains including Salmonella (Abdellah et al., 2009).

Results of sensitivity and suscepility of bacterial isolates against used 16 antibiotics (Table 3). It was observed that no single antimicrobial drug was 100% effective against different isolates. It is clear that most isolates were resistant to Tetracycline, Oxytetracycline, and Erythromycin. This may be due to development of resistance which attributed to the large scale indiscriminate use of antimicrobials as feed additives and also misuse for therapeutic purposes. More or less the same findings were reported by Ramaswamy et al. (1982) and Sinha et al. (1985). The present study showed highest sensitivity to Quinolone (NorfLoxacin and Ciprofloxacin) may be due to these drugs are recently introduced, have broad spectrum of action and limitedly used so far, by the poultry farmers. The results correlated with that of Veere Gowda et al. (1996).

The phenomenon of drug resistance among chickens isolates is of clinical significance because these organisms may be transferred to produce human infections specially if we know that Antibiotic resistance has reached crisis point in many hospitals around the world.. The use of antimicrobial drugs as growth promoters and to the recommendation for restriction of their use for treatment of infections in the chickens industry Van den Bogaard and Stobberingh (1996). The sensitivity test is of great value in recommendation of the effective antibacterial agents also prevention of microbial resistance, and so the cost of combating bacterial infections can be significantly minimized.

In conclusion our study showed that the possible role of such live poultry markets in spreading of poultry and human pathogens, to environment as well as to the drainage system cannot ignored, where the hygienic measures were not adopted in such shops. This needs more attention by authorities to take these sources of microbial infections in mind; moreover most of the isolated organisms had alarmingly high rates of resistance to at least12 antimicrobials. We recommend the strict application of sanitary measures in life poultry markets to minimize bacterial contamination and spread of pathogens; especially to human. This study will be followed by studies on pathogenicity of the obtained isolates to chickens.

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معدلات التلوث البكتيرى فى مخلفات الزبح فى اسواق الدواجن الحية الخاصة فى مدينة صنعاء.

١- عزل وتصنيف وحساسة المعزولات للمادات الحيوية.

غازى على زهير

كلية الزراعة جامعة صنعاء جمهورية اليمن

أجريت هذه الدراسة لاستبان معدلات التلوث البكتيرى لمخلفات الزبح فى اسواق الدواجن الحية (المحلات) فى مدينة صنعاء. تم الفحص البكتيرى لعدد ١٤٦ عينة اشتملت على ٦٣ عينة امعاء و ١٧ عينة مياة من خزانات الغسيل و ١٩ عينة من الرش و ٢٥ عينة طحال و ٢٢ عينة عظام طويلة نتج عنها ٣٥ , ١١ , ٥ , ١ , ٠ معزولة بكتيرية وبنسب عزل 55.5 , 64.7 , 26.3 , 4 , ٠ % ; على التوالى.

تم التعرف على العترات المعزولة بالوصف الشكلى للمستعمرات , صبغة الجرام , التفاعات الكيمائية الحيوية لعدد ٥٢ معزولة من العينات بمعدل كلى 35.6% وتم تعرفها الى 27 (51.9%) معزولة من الميكروب القولونى , 10 (19.3%) معزولة من السيتروباكتر فروندى , 3 (5.7%) كليبسيلات نمونى , 1 (1.9%) من السلمونيلا انترتدس ,3 (3.8%) كمبيلو باكتر جوجوناى ,1 (1.9%) سدوموناس ايروجينوزا , ٥ المكور العنقودى موجب الكواجيوليز , ٣ (5.7%) المكور السبحى المحلل للدم من النوع البيتا.

اوضح اختبار حساسية المعزولات للمضادات الحوة الى انة لم يسجل اى من المضدات المستخدمة وعددها ١٦ نوعا احدث اجابية بمعل ١٠٠ من اى من المعزوات المختبرة. معضم المعزولات كانت مقاومة لعقارات التيتراسيكليين , الاوكسيتيتراسيكلين , الارثرومايسين.

من النتائج نوصى بضرورة تطبيق الاجرائات و الاشتراطات و الصحية الصارمة فى اسواق الدواجن الحية ومجازرها حد من التلوث البكتيرى و نشر الميكروبات الممرضة بصفة خاصة للانسان. سوف تخضع المعزولات اتى تم الحصول عليها من هذه الدراسة الى اختبار ضراوتها لكتاكيت دجاج عمر يوم واحد.