BACTERIAL ORGANISMS ISOLATED FROM HEALTHY CHICKEN IN THE EASTERN PROVINCE OF SAUDI ARABIA AND THEIR PATTERN OF RESISTANCE TO ANTIMICROBIAL AGENTS

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ABSTRACT:

The antibiogram of normal bacterial flora isolated from chicken farms over a two years period was investigated in the Eastern Province of Saudi Arabia. Of the 28 types of bacterial organisms isolated, E.coli was the most common (16.2%) followed by S. saprophyticus (13.9%), S. epidermidis (9.7%) *Proteus spp* (6.9%), *Enterococcus spp* (5.2%) and *Klebsiella spp* (3.7%).

The rank order of resistance observed in this study was spectinomycin (89.9%), tetracycline (89.2%), penicillin (80.9%), erythromycin (72%), ampicillin (52.9%) and the combination of trimethoprim and sulphamethaxazole (48.4%). High resestant rate (43.0%) to chloramphenicol which is not used in chickens production was also observed. This could be associated with the misuse of spectinomycin.

Although the normal flora were not affected by the type of farm design, location or type of product, the pattern of resistance showed some significant differences which may reflect the extent of use of these drugs.

It is concluded that in chickens, bacterial resistance to antimicrobial drugs is alarmingly high in the eastern province of Saudi Arabia. This may suggest that their use is not cost-effective as prophylactic against infections or as growth promoters. It also stresses the need for stricter regulations for the use of antimicrobial drugs in the chickens industry.

Keywords: Antimicrobials, Bacterial resistance, Chickens, Normal flora, Saudi Arabia

INTRODUCTION:

The extensive use of chemotherapeutic agents to improve chickens production is a phenomenon of our time. Large numbers of antimicrobial agents are being used, often concomitantly, for treatment and prevention of chickens infections (Swann report, 1969; Smith, 1974; Aronson, 1975). This practice may stimulate bacterial resistance to antimicrobial agents which would complicate the treatment of animals and, possibly human infections as a result of the transfer of resistance from animal to human pathogens (Aronson, 1975; Mercer, 1975; Holmberg et al., 1984). In addition, allergic symptoms (Woodward, 1991) as well as disturbances of intestinal flora in humans may be caused by antimicrobial residues in food (Holmberg et al., 1984). It is reported that feeding of tetracycline-containing diets resulted in replacement of the non-pathogenic E.coli in the alimentary tract of chickens by tetracycline resistant E.coli (Smith, 1974).

Antimicrobial agents are widely used in the chickens industry in Saudi Arabia. Our own survey showed that 28 agents are available for chickens use in the Eastern Province of Saudi Arabia (unpublished). These include β-lactams, tetracyclines, macrolides, aminoglycosides, fluoroquinolones, lincosamides and sulphonamides. They are mainly added to drinking water for infection prophylaxis. In addition, it was noted that 20 of these antimicrobial agents were also commonly used in the treatment of human infections (unpublished). However, no studies have been carried out in Saudi Arabia to evaluate resistance to antimicrobials in chickens and its possible impact on human health.

The aim of this study was to identify organisms isolated from healthy chickens in the Eastern Province of the Kingdom, and to study their pattern of resistance to the commonly used antimicrobial agents in chickens industry as well as the ones most recommended for human pathogens.

MATERIAL AND METHODS:

Twenty-three out of 87 (30 in Dammam and 57 in Alhasa) chickens farms in the eastern province of Saudi Arabia were randomly selected for the study. Location of the selected farms (9 in Dammam and 14 in Alhasa), design (17 open and 6 closed system) and type of product (16 broiler and 7 layer) were taken into consideration. The selected farms were visited at least 3 times between 1st January 1995 and 31st December 1996.

Ten apparently healthy chickens were randomly selected in each visit and swabs were taken from their skin and rectum as well as from the egg-yolk. The swabs were directly inoculated on Mac-Conkey medium, eosine-methylene blue medium (EMB) for primary isolation of all species of gram-negative bacilli. Rectal and egg swabs were also directly inoculated on xylose lysine desoxycholate (XLD) agar plate for selective screening of Salmonella and *Shigella Spp*. A small proportion of the specimen was enriched by inoculation on selenite-F broth and incubated for 24, then subculture was made on Hektoen enteric (HE) agar. Skin swabs were inoculated on nutrient agar, mannitol salt agar and blood agar while bile esculin and campylobacter agar media were used for isolation of enterococci and campylobacter, respectively. All plates were incubated at 35^{o}C for 24-48 hours. For anaerobic organisms, incubation was made under anaerobic conditions by use of Gas-Pak system (BBL, Becton Dickinson Co., Cockeysville, MD, USA.) in the anaerobic jar for 72 hours. The isolated organisms were tested against antimicrobial drugs used on the farm as well as the antimicrobial agents most recommended for treatment of human infections. The single-disc antibiotic-sensitivity testing method was used throughout this study. Susceptibility tests were done using BBL® Sensi-Disc® antimicrobial susceptibility test discs (Becton Dickinson). The criteria of interpretation were those recommended by the National Committee for Clinical Laboratory Standards (Anon, 1993) and recommended quality control procedures were performed on a regular basis. All results were recorded and later entered in a database for analysis using SPSS/ PC software.

RESULTS:

A total of 3529 specimens were inoculated of which 881 (25%) showed no growth. These were mainly from egg yolk specimens. The remaining 2648 (75%) specimens showed positive growth. The most frequently isolated organisms were E. coli (16.2%), S. saprophyticus (13.9%), S. epidermidis (9.7%), *Proteus spp.* (6.9%), *Enterococcus spp* (5.2%), *Klebsiella spp.* (3.7%), *Bacillus spp*.(3.3), *Pseudomonas spp*. (2.8%), *Micorcoccus spp*. (2.6%), and others (10.9%). Farm's location, type of production and design had no significant effect on the prevalence of the isolated organisms.

The overall resistance rates of isolated organisms to 28 types of antimicrobial agents are illustrated in Table 1. High rates of resistance (> 35 %) were observed with spectinomycin, tetracycline, penicillin, neomycin, doxycycline, erythromycin, ampicillin, chloramphenicol, colistin, trimethoprim + sulphamethaxazole (TMP + SMX), nitrofurantoin and gentamicin. On the other hand, low rates of resistance (<15 %) were noted with amikacin, pipracillin, amoxicillin + clavulanate (AMX + CLV), imipenem, vancomycin, kitassamycin and streptomycin (Table 1).

The resistance rates of penicillin, doxycycline, lincomycin and neomycin were found to be significantly higher ($p \leq 0.0002$) in organisms isolated from Al-Hassa-farms than in those isolated from Dammam. On the other hand, rates of resistance to ciprofloxacin, gentamicin, TMP + SMX and nitrofurantoin were found to be significantly higher ($P \leq 0.004$) among organisms isolated from Dammam than in those from Al-Hassa region.

Resistance rates of organisms isolated from broiler chickens to norfloxacin, gentamicin and neomycin were significantly higher ($P= 0.0000$) than those of organisms isolated from layer chickens. In contrast, rates of resistance to piperacillin, ceftazidime, erythromycin and colistin were found to be significantly higher ($P\leq 0.025$) among organisms isolated from layers when compared with those isolated from broilers.

The overall resistance rate to Spectinomycin was (89.9%) with all tested organisms being highly resistant to this drug (Table 2). Similarly, high resistance rates to tetracycline (89.2%) were also observed (Table 3).

Sensitivity of 1493 isolates to penicillin is presented in Table 4. The overall resistance rate was (80.9%) with the highest rates being 99.6% observed with *Proteus spp.*, followed by enterococci, S. saprophyticus, and S. epidermidis. In contrast, the overall resistance rate of 2242 isolates to ampicillin was (52.5%) with E. coli being the most resistant (98.5%) to this drug (Table 5).

The overall resistance rate to erythromycin was 70.2% with E. coli showing the highest rate (98.5%) followed by S. saprophyticus, and S. epidermidis (Table 6).

The overall resistance rate of 2050 isolates to TMP +SMX (Septrin) was 48.4%. The highest resistance rate (97.9%) was that of *Proteus spp* followed by that of E. coli (Table 7).

Sensitivity of 1948 isolates to chloramphenicol was investigated. The overall resistance rate was 43.4% with E. coli showing the highest rate (54.5%) followed by S. epidermidis, and S. saprophyticus (Table 8).

The overall resistance rate of 530 isolates to nitrofurantoin was 39.6%. This drug was mainly tested against E. coli organisms, which showed a resistance rate of 33.6% (Table 9). Similarly, the overall resistance rate of 2469 isolates to gentamicin was 37.4% (Table 10). However, E. coli had the highest resistance rate (84.7%) followed by *Pseudomonas spp.*, *Proteus spp.* and *Klebsiella spp* .

DISCUSSION:

This study was conducted to identify the common bacterial organisms in locally produced healthy chickens. The most frequently isolated organisms were E. coli, S. saprophyticus, S. epidermidis, *Proteus spp*, *Enterococcus spp, Klebsiella spp.*, *Bacillus spp.*, *Pseudomonas spp.* and *Micorcoccus spp*. Although, most of the isolated organisms are considered normal flora, some like *Salmonella spp.* (0.1%) are primary pathogens. Neither farm's site nor type of its product or design affected the prevalence of these organisms.). In a similar study, E.coli, *Citrobacter spp.*, *Proteus spp.*, *Enterobacter spp.*, *Klebsiella spp.* and P. aeruginosa were found to be the most isolated organisms from 152 apparently healthy ducks Aora et al. (1988).

The make-up and prevalence of normal flora are usually influenced by many factors such as balance between various types of organisms as well as the antimicrobial agents added to chickens feeds. If disturbed, the flora may cause disease to animals (Jawetz et al., 1984). Furthermore, packing and storage conditions of slaughtered chickens may also alter the prevalence of these organisms (Studer et al., 1988; Muller-Hohe, 1989.

In the chickens industry, antimicrobial drugs are mainly used either as feed additives for nutritional purposes or as prophylactics against stress related infections. The mechanisms by which antimicrobial drugs promote animal growth are not fully understood (Prescott & Baggot, 1993a). For these reasons, in this study, the antibiograms of isolated organisms in each farm were investigated against antimicrobials used in that farm regardless of the drugs antimicrobial spectrum. The results indicated that the resistance rates to penicillin, doxycycline, lincomycin and neomycin were significantly higher among organisms isolated from Al-Hassa-chicken farms compared with those isolated from Dammam, while the reverse was observed with ciproflxacin, gentamicin, TMP + SMX and nitrofurantoin. This could be related to differences in the extent of dispensing and use of antimicrobial drugs in these regions. Indeed, the highest rates of resistance observed in this study were those of heavily used agents such as spectinomycin and tetracycline. Moreover, it is well documented that these two drugs antagonize the effect of each other (Allen et al., 1993), which suggests that, their concomitant use is scientifically baseless.

Penicillin G is used in the chickens industry for the prevention and treatment of necrotic enteritis and ulcerative enteritis (Prescott & Baggot, 1993b). The overall resistance to this drug was extremely high (> 80%) which was not unexpected since most organisms were either gram negative or beta lactamase producers. This again confirms the irrational use of this drug in chicken production.

Erythromycin has good activity against gram-positive and some gram negative aerobes (Prescott & Baggot, 1993c). In chickens, the drug is administered in water for the prevention and treatment of staphylococcal or streptococcal infections, necrotic dermatitis and infectious coryza. However, very high resistance rates (>70%) to this drug were observed with most investigated organisms. The resistance may either be natural such as that in E. coli or acquired possibly due to cross-resistance with lincosamides (Von Recklinghausen et al., 1989). Indeed, lincomycin is heavily used in the investigated farms and may therefore, contribute to this high rate of resistance.

Despite the unavailability of chloramphenicol for chicken use in the Kingdom, moderate to high resistance rates (15 - 54 %) to this drug were observed. This could be associated with the misuse of spectinomycin as was prevoiusly reported by Ginns et al. (1996). The banning of chloramphenicol in food producing animals was due to the potential risk of dose-independent fatal aplastic anemia in humans (Settpani, 1984). In addition, acquired resistance in some enterobacteriace could be transmitted to human pathogens (Spika et al, 1987, Anon 1976).

Gentamicin is heavily used in chicken farms, it is administered to almost all 1-3 day old chicks as a prevention or treatment of E. coli, P. aeruginosa and *salmonella spp*. infections (Rhoades, 1979). It is rather unfortunate, that most gram- negative organisms in this study were highly resistant to this drug. However, this is in agreement with the known pattern of resistance to gentamicin, which is more common in veterinary hospitals than in the community (Koterba et al., 1986).

Moderate resistance rates were observed with TMP+SMX in most organisms except E. coli strains (84.7%). Similarly, Blanco et al (1997) in Spain showed that up to 67% of avian E. coli strains were resistant to this combination, and that resistance to the new fluoroquinolones was also increasing. This again concurs with our results, which showed moderate resistance to ciprofloxacin and norfloxacin despite their relatively recent introduction to the Saudi market.

Our results clearly demonstrated that normal flora isolated from chickens were highly resistant to most of the growth-promoter antimicrobial drugs approved for use in the United States or European Union (Prescott & Baggot, 1993a). This may reduce the economic value of these drugs as growth promoters. Indeed, Hayes (1981) reported the decline of growth promotion response of pigs to antimicrobial agents associated with increased resistance to the used agents.

In conclusion our study showed that most of the isolated organisms had alarmingly high rates of resistance to at least 12 antimicrobials. The degree of resistance to various antimicrobials shown by different types of isolates may be related to the extent of use or exposure to these agents. The phenomenon of drug resistance among chickens isolates is of clinical significance because these organisms may be transferred to produce human infections (Martel & Coudert, 1993; Nair et al., 1995; Gaunt & Piddock, 1996, Al-Ghamdi et al., 1999). This study, therefore, lends support to the calls for banning 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 & Stobberingh, 1996).

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Table (1) : Patterns of resistance to antimicrobial drugs by organisms isolated from chicken farms.

Antimicrobial's Name	No. Tested	Sensitive	Resistant	% Resistant
Amikacin	103	89	14	13.6
Amoxycillin	72	30	40	58.3
Ampicillin	2242	1055	1187	52.5
Augmentin	1001	910	91	9.0
Aztreonam	118	96	22	18.6
Ceftazidime	1913	1600	313	16.4
Cefuroxime	11	9	2	18.2
Chloramphenicol	1581	1086	862	54.5
Chlorotetracycline	33	5	28	84.8
Ciprofloxacin	354	230	123	34.7
Colistin	200	97	103	51.5
Doxycycline	273	81	192	70.3
Erythromycin	1391	415	976	70.2
Gentamicin	2469	1546	923	37.4
Imipenem	330	312	18	5.4
Kitassamycin	49	47	2	4.1
Lincomycin	120	100	20	16.7
Neomycin	172	41	131	76.2
Nitrofuration	530	320	210	39.6
Norfloxacin	385	298	87	22.6
Pencillin	1493	285	1208	80.9
Piperacillin	760	667	93	12.2
Spectinomycin	910	92	818	89.9
Streptomycin	31	30	1	3.2
Sulfamethoxazole &	2050	1057	993	48.4
Tetracycline	1962	212	1750	89.2
Vancomycin	179	177	2	1.1

Table (2) : Pattern of resistance to spectinomycin

Organisms	No. tested	Sensitive	Resistant	% Resistance
E. coli	553	28	525	94.9
Klebsiella spp	126	24	102	81.0
B. fragilis	38	00	38	100.
Campylobacter spp	40	17	23	57.5
Others	153	23	130	85.0
Total	**910**	**92**	**818**	**89.9**

Table (3) : Pattern of resistance to tetracycline

Organisms	No. tested	Sensitive	Resistant	% Resistance
E. coli	563	5	558	99.1
S. saprophyticus	482	53	429	89.2
S. epidermidis	335	50	285	85.1
Klebsiella spp	123	3	120	97.6
Bacillus spp	111	36	75	67.6
Micrococcus	92	46	46	50.0
Others	256	19	237	92.6
Total	**1962**	**212**	**1750**	**89.2**

Table (4) : Pattern of resistance to Penicillin

Organisms	No. tested	Sensitive	Resistant	% Resistance
S. epidermidis	329	84	245	74.5
S. saprophyticus	470	97	373	79.4
Enterococcus	178	18	160	89.9
Proteus spp	238	1	237	99.6
Micrococcus spp	91	47	44	48.4
Others	187	38	149	79.7
Total	**1493**	**285**	**1208**	**80.9**

Table (5) : Pattern of resistance to ampicillin

Organisms	No. tested	Sensitive	Resistant	% Resistance
E. coli	565	104	461	81.6
S. saprophyticus	484	331	153	31.6
S. epidermidis	333	251	82	24.6
Klebsiella spp	127	100	27	21.3
Bacillus spp	111	81	31	27.9
Micrococcus spp	92	87	5	5.40
Bacteroides fragilis	38	7	31	81.6
Others	492	94	397	80.7
Total	**2242**	**1055**	**1187**	**52.9**

Table (6) : Pattern of resistance to erythromycin

Organisms	No. tested	Sensetive	Resistant	% Resistance
E. coli	66	1	65	98.5
S. saprophyticus	478	109	369	77.2
S. epidermidis	334	80	254	76.0
Enterococcus spp	161	47	114	70.8
Bacillus spp	112	63	49	43.8
Micrococcus spp	92	65	27	29.3
Others	148	50	98	66.2
Total	**1391**	**415**	**976**	**70.2**

Table (7) : Pattern of resistance to trimethoprim + sulphamethaxazole (TMP + SMX)

Organisms	No. tested	Sensitive	Resistant	% Resistance
E. coli	562	86	476	84.7
S. saprophyticus	483	332	151	31.3
S. epidermidis	337	254	83	24.6
Klebsiella spp	125	55	70	56.0
Proteus spp	97	2	95	97.9
Bacillus spp	111	107	4	3.60
Micrococcus spp	92	91	1	1.10
Others	243	130	113	46.5
Total	**2050**	**1057**	**993**	**48.4**

Table (8) : Pattern of resistance to chloramphenicol

Organisms	No. tested	Sensitive	Resistant	% Resistance
E. coli	560	255	305	54.5
S. saprophyticus	478	249	229	47.9
S. epidermidis	328	158	170	51.8
Klebsiella spp	125	87	38	30.4
Bacillus spp	108	60	48	44.5
Micrococcus spp	92	78	14	15.2
Others	257	199	58	22.6
Total	**1948**	**1086**	**862**	**43.4**

Table (9) : Pattern of resistance to nitrofurantoin

Organisms	No. tested	Sensitive	Resistant	% Resistance
E. coli	360	239	121	33.6
Proteus spp	39	3	36	92.3
Bacteroides fragilis	39	34	5	12.8
Others	92	44	48	52.2
Total	**530**	**320**	**210**	**39.6**

Table (10) : Pattern of resistance to gentamicin

Organisms	No. tested	Sensitive	Resistant	% Resistance
E. coli	562	86	476	84.7
S. saprophyticus	484	425	59	12.2
S. epidermidis	337	275	62	18.4
Proteus spp	236	97	139	58.9
Enterococcus spp	176	137	39	22.2
Klebsiella spp	126	59	67	53.2
Bacillus spp	110	97	13	11.8
Micrococcus spp	92	91	1.0	1.10
Pseudomonas spp	92	30	62	67.4
Others	254	249	5.0	2.00
Total	**2469**	**1546**	**923**	**37.4**

جراثيم البكتيريا المعزولة من دواجن معافاة بالمنطقة الشرقية من المملكة العربية السعودية ونمط مقاومتها للمضادات الحيوية

***مستور بن سفر الغامدي ، فكري ا رسي**

****زكي بن حسن ا صطفي ، مصطفى فايز ، مصطفى الرمضان**

***قسمي علم الأدوية والأحياء الدقيقة، كلية الطب وكلية الطب البيطري**
جامعة الملك فيصل، الدمام المملكة العربية السعودية

ا لمخص :

تع هذه الدراسة بنمط حساسية البكت يا ا عزولة من الدواجن ا نطقة الشرقية من ا ملكة العربية السعودية للمضادات ا يوية. وقد ت الدراسة خلال عام إبتداءا من يناير عام ١٩٩٥ م و فيها عزل ٢٨ صنفا من البكت يا وكانت عصويات ش يشيا القولون تشكل النسبة الك ى منها (٢و١٦%) تليها كل من ا كور العنقودي الرمي (٩و١٣ %) وا كور العنقودي ا لمدي (٧و٩ %) وعصويات ال وتيس (٩و٦ %)وا كور السبحي ا عوي (٢و٥ %) وعصويات الكلبسيلا (٣و٧ %).

وحسب الترتيب التناز للمقاومة البكت ية للمضادات ا يوية وجد أن ٩و٨٩ % من البكت يا مقاوم للسبكتينومايس ، ٢و٨٩ % للتتراسيكلا ، ٩و٨٠ % للبنسلا ، ٧٢% للأرثرومايس ، ٩و٥٢ % للأمبسللا ، ٤و٤٨ % للكوتراميكسازول (دواء مركب من سلفامثيكسازول و ترا يثوبر). كما أشارت الدراسة إ مقاومة عالية للكلورامفنيكول (٤٣%) علما بأن هذا الدواء لا يستخدم إنتاج الدواجن وقد يكون ذلك نتيجة لسؤ استخدام الأسبكتينومايس .

وبالرغم من أن أصناف النبت ا رثومي ا عزول من الدواجن تكن متأثرة بتصميم ا زرعة (مغلق أو مفتوح) ولا موقعها (الدمام أو الأحساء) أو نوع ا نتج (دجاج لاحم أو بيّاض) إلا أنه لوحظ وجود فوارق ذات دلالة إحصائية معدل مقاومة البكت يا للمضادات ا يوية ا قد يعكس مدى استخدام هذه الأدوية تلك ا زارع.

ويستنتج من هذه الدراسة بأن ا قاومة البكت ية للمضادات ا يوية الدواجن ا نتجة با نطقة الشرقية من ا ملكة قد بلغت معدلات تنذر با طر، ا د من الفائدة ا رجوة من استعما ا الوقاية أو العلاج من الأمراض الوبائية أو لتحفيز النمو و عل استعما ا عد ا دوى من الناحية الإقتصادية. كما أن هذه الدراسة تؤكد ا اجة إ سن نظام ملائم يقنن استعمال ا ضادات ا يوية و د من سؤ استخدامها إنتاج الدواجن.

الكلمات الدالة : ا ضادات ا يوية، ا قاومة البكت ية، الدواجن، النبت ا رثومي، ا ملكة العربية السعودية