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ANTIBIOTIC RESISTANCE AND PLASMID PROFILE OF *ESCHERCHIA COLI* AND *STAPHYLOCOCCUS AUREUS* ISOLATED FROM LUNCHEON

(With 6 Tables & 1 Figure)

By
R.S. REFAIE; H.H. ESSA; M.M. ALI. and AMAL. A. MOHAMMED

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مقاومة بعض المضادات الحيوية وتوصيف البلازميدات لميكروبى القولوني والمكور العنقودي الذهبي المعزولين من اللانشون

رمضان سيد رفاعى ، حمدى حسين عيسى ، محمد مصطفي على ،
آمال أحمد محمد

نظرا لأهمية وخطورة البلازميدات المقاومة البكتيرية للمضادات الحيوية ومدى انتقالها من ميكروب إلى ميكروب وبالتالي للإنسان. فقد تناولت هذه الدراسة فحص ٥٤ عينة من اللانشون التي تم جمعها من محلات مدينة أسيوط المختلفة, أظهرت النتائج عن عزل ١٥ عترة من الميكروب القولونى وبالفحص السيرولوجي لهم تبين أن عترتين هما $O_{114}\ K_{90}$, $O_{26}\ B_6$ والباقي سلبي. كما تم عزل ٢٥ عترة من الميكروب المكور العنقودي الذهبي. تم إجراء اختبارات الحساسية لعدد ١٠ مضادات حيوية (جنتاميسين , ايرثرومسين , كلورامفينكول وأموكسيل , اميكاسين, أمبيلكوكس , حامض الناَلدكسيك , اوكسى تتراسيكلين, بنسلين , توبراميسين) . وقد أسفرت النتائج عن قدرة الميكروب القولونى للمقاومة العالية ضد المضادات الحيوية مثل البنسلين و أمبيلكوكس وأموكسيل بنسب ١٠٠ و ٩٣,٣ و ٩٣,٣% على التوالي. كذلك قدرة الميكروب المكور العنقودي الذهبي للمقاومة العالية ضد المضادات الحيوية مثل البنسلين و أمبيلكوكس وأموكسيل بنسب ١٠٠ و ٩٦ و ٧٢% على التوالي. وبدراسة تعدد المقاومة البكتيرية للميكروب الواحد أسفرت النتائج عن وجود نسب ٦,٧ و ٢٠ و ٢٦,٧ و ٤٠ و ٦,٧% من كل عترات الميكروب القولونى المختبرة يقاوم مقاومة متعددة لعدد ٢ و ٣ و ٤ و ٥ و ٦ من المضادات الحيوية على التوالي. كذلك وجود نسب ٤ و ١٢ و ٤٠ و ٤٠ و ٤% من كل عترات الميكروب المكور العنقودى الذهبى المختبرة يقاوم مقاومة متعددة لعدد ١ و ٢ و ٣ و ٤ و ٦ من المضادات الحيوية على التوالي. بالبحث عن وجود البلازميدات في عترات الميكروب القولونى المعزولة من اللانشون بالتحليل الالكتروفوريسى للبلازميدات المستخلصة وجد انها تتروا‌ح بين (١-٣) بلازميدات في العترة الواحدة باحجام جزيئية مختلفة تتراوح بين (١١-٥٣) كيلوبيز. كما

أسفرت النتائج على عدم وجود أى من البلازميدات في عترات الميكروب المكور العنقودي الذهبي.

SUMMARY

Fifty four samples of luncheon meat were collected from Assiut City supermarkets and shops and examined bacteriologically. 15 (27.8%) *E.coli* were detected in the examined luncheon samples, two serotypes of Enteropathogenic *E.coli* (EPEC) were recorded (**O_{26} B_6 , O_{114} K_{90}**)and the other were untypable. 25 (46.3%) *Staph. aureus* were detected from the examined samples. All isolates were tested to antibiotic resistant against gentamycin, erythromycin, chloramphenicol, amoxil, amikacin, ampiclox, nalidexic acid, oxytetracyclin, penicillin and tobramycin. *E.coli* isolates showed high antibiotic resistance against penicillin, ampiclox and amoxil with percentages 100, 93.3 and 93.3% respectively. *Staph.aureus* isolates showed high antibiotic resistance against penicillin, ampiclox and amoxil with 100, 96 and 72% respectively. Multidrug resistance study revealed that 6.7, 20, 26.7, 40 and 6.7% of the tested *E.coli* isolates resisted double, triple, quadruple, quintuple and sixtuple antibiotic agents respectively. Multidrug resistance revealed that 4, 12, 40, 40 and 4% of the tested *Staph.aureus* isolates resisted single, double, triple, quadruple and sixtuple antibiotic agents respectively. Screening for plasmid presence using electrophoresis analysis revealed that the extracted plasmid contents were (1-3) plasmid DNA per a single *E.coli* isolates with molecular size ranged from 11 up to 53 kilo base. All *Staph.aureus* isolates were negative for plasmid profile analysis.

Kery words: *Microorganisms – Antibiotic resistance – plasmid profile analysis.*

INTRODUCTION

Recently, Piddock (1996) proposed three possible scenarios by which the use of antibiotics in food animals could pose a risk to human health: 1) Antibiotic-resistant bacteria pathogenic to humans are selected, and food is contaminated during slaughter and/or preparation. When the food is ingested, the bacteria cause a infection that requires antibiotic treatment, and therapy is compromised. 2) Antibiotic-resistant bacteria nonpathogenic to humans are selected in the animal. When the contaminated food is ingested, the bacteria transfer the resistance to other bacteria in the human gut. 3) Antibiotic remain as residues in animal products, which allows the selection of antibiotic-resistant bacteria in the consumer of the food. The danger of this to humans is that antibiotic-resistant non-pathogenic organisms in an animal may be passed to, and colonize humans, carrying plasmids into the human environment, these plasmids may subsequently be transferred to human pathogens or to indigenous flora in the human body (Levy 1992).

Bacteria are carrying genes encoding for antibiotic resistance which called plasmids replicate autonomously (Wagner and Hahn 1999). Many new antibacterial drugs have been developed and used. Plasmids conferring resistance to them have often appeared within a short time.

The practical importance of plasmid-determined genes is recognized with the discovery of transferable drug resistance (Datta and Nagent 1984). This transmission of drug resistance is occured when plasmids or bacterial strains containing plasmids are transmitted between different animals even of different species (Chaslus-Dancla *et al.,* 1987). Rather than the potential cross infection of human beings from animals and vice versa via such plasmids (Singh *et al.,* 1992).

Therefore, the present study was conducted to:

- Define the antibiotic resistance of *E.coli* and *Staph. aureus* strains isolated fom luncheon meat.
- Perform plasmid profile analysis of these multidrug-resistant strains.

MATERIAL and METHODS

Collection of samples:

Fifty-four random samples of luncheon meat were collected from Assiut City supermarkets and shops. The samples were transferred as quickly as possible to the laboratory without delay to be immediately subjected for bacteriological examination.

Isolation and identification of *E.coli*:
were carried out according to (ICMSF, 1978). The slide agglutination technique (ICMSF, 1978) was adopted for serotyping of *E.coli* strains.

Isolation and identification of *Staph.aureus:*

Two gms. from each sample were inoculated into trypticase soy broth tubes and incubated at 37°C for 24 hours. (A.P.H.A., 1992). Loopfuls from incubated tubes were streaked on Baird-Parker medium which is highly recommended and excels all other media (I.C.M.S.F., 1978). Isolated strains were subjected to morphological and biochemical examination according to (A.P.H.A., 1992 and I.C.M.S.F, 1996).

Antibiotic resistance technique:

Disc diffusion method (DD): The standardized strain suspension was prepared as the staphylococci isolates were suspended in trypticase soy broth for 18 hrs, then the suspension was adjusted to a turbidity equivalent to 0.5 McFarland standard by adding sterile saline, then it was suitable for sensitivity testing by DD procedure (Quinn *et al.*, 1994). The entire surface of a trypticase soy agar plate was streaked by a sterile cotton swab soaked in the standardized tested strain suspension. After complete drying, sensitivity antibiotic discs were gently placed [gentamycin 10 µg, erythromycin 15 µg, chloramphenicol 30 µg, amoxil 25 µg, amikacin 30 µg, ampiclox 30 µg, nalidexic acid 30 µg, oxytetracycline 30 µg, penicillin 10 I.U and tobramycin 10 µg - Oxoid Limited-England]. The test was performed according to Bauer *et al.* (1966). Categorizing the tested strains to susceptible or resistant was judged by measuring the whole inhibition zone ∅ according to Bauer-Kirby Scale (Atlas, 1995).

Plasmid profile analysis:

Extraction of plasmid DNA:

Two typable *E.coli* isolates represnting O26 B6 and O114 K90 serotypes beside three untypable and five Staph.aureus isolates were grown overnight at 37 °C in Luria-Bertani medium (LB medium) with shaking. Plasmid DNA was extracted from the bacteria using alkaline lysis method after Bironboim and Doly (1979). The concentration and purity of the extracted DNA was determined by spectrophotometry (Gene Quant II, pharmacia Biotech.)

Agarose gel electrophoresis:

The extracted plasmid DNA was analysed by electrophoresis on 0.7% agarose gels at 120 volts for 1.5 hours and stained with 0.5 µg/ml of ethidium bromide. 2 µl of RNase (20 mg/ml) was included in the electrophoresis for each sample as well as DNA molecular size marker. The gels were photographed under UV transilluminator (Biometer).

RESULTS

Results were explained in Tables 1-6 and 1 Figure.

DISCUSSION

The development of some resistance by bacterial pathogens is almost certainly an inevitable consequence of the clinical use of antimicrobial drugs. Excessive use of antibiotics for treating animal diseases and subtherapeutic applications of antimicrobial agents for disease prevention, growth promotion, and feed efficiency in livestock and poultry production also have accelerated the emergence of antibiotic-resistant bacteria, which can then be transferred to humans through the food chain (Meng *et al.*, 1998).

The public health hazard of *E.coli* strains when they are present in human gut is manifested not only in being facultative pathogens, but also in disseminating their multidrug resistance plasmids and infecting other microorganisms since the transference of these plasmids can take place and the exchange may occurs even between gram +ve and gram -ve bacteria through conjugational genetic transfer (Mazodier and Davis, 1991 and Kessie *et al.*, 1998).

The incidence of *E.coli* organisms in the examined luncheon meat was 15 (27.8%) (Table 1). These findings were in acceptance with that recorded by Eman (1990) and Youssef *et al.* (1999). lower incidence of *E.coli* in luncheon samples were reported by Ahmed (1992) and Youssef *et al.*, (1999). The increase incidence of *E.coli* in the examined samples may be due to mishandling during production, processing and distribution. Two *E.coli* serotypes were Enteropathogenic *E.coli* (EPEC) (O26 B6 and O114 K 90) (13.3%) could be isolated from the examined samples and the other *E.coli* isolates 13 (86.7%) were untypable (Table 1), Eman (1990), Darwish *et al.* (1991) and Youssef *et al.* (1999) could isolate different serovars from meat products. *E.coli* constitutes a public health importance and one of the food poisoning agents (Beckers 1986). Meat products were implicated in two separate outbreaks of food poisoning due to Enteropathogenic *E.coli* (Doyle and Padyhe, 1989).

It is evident from the recorded data (Table 1) that *Staph.aureus* could be detected in 46.3% of the examined samples. *Staph.aureus* existed in many of the examined meat products, some of them were contaminated with high level of such organisms and this may be due to its spread by food handlers, particularly of infected wounds or sores on their hands (Mahoney and Compbell, 1983 and Youssef *et al.,* 1999).

In the present investigation 100, 93.3 and 93.3% of the examined *E.coli* strains were highly resistant to the action of penicillin, ampiclox and amoxil respectively, (Table 2). In recent years, there is an alarming increase in the rate of human infections with antibiotic resistant microorganisms (Salvat *et al.* 2001). Two main categories of bacteria carrying genes encoding for antibiotic resistance which may be transmitted from animals to humans via food products. The first category is the obligate infectious pathogens as *Salmonella enterica*, while the second one is the facultative pathogenic species as *E.coli* (Wagner and Hahn, 1999).

Table (3) shows that most of the examined *Staph.aureus* strains were highly resistant to the action of penicillin, ampiclox and amoxil with an incidence of 100, 96 and 72% respectively. Staphylococcal penicillin resistance was widly established. Costa *et al.* (1996); Andrade *et al.* (2000); Younis *et al.* (2000); and Viera *et al.* (2000) reported that penicillin resistance percentages were 100, 97, 86 and 87% respectively. The principle limitation of penicillin usage is its susceptibility to be destructed by β- lactamase enzyme (pencillinase) produced by many staphylococcus spp. (Watts and Salmon, 1997).

The recorded data in Table (4) revealed that, all tested *E.coli* strains were multidrug-resistant (6.7% were double, 20% were triple, 26.7% were quadruple, 40% quintuple and 6.7% were sixtuple antibiotic resistant). Multidrug resistance were widely studied. Cid *et al.* (1996) showed that 55% were quadruple and 33% were sixtuple antibiotic resistant strains. Urassa *et al.* (1997) reported that more than 80% of the tested strains were resistant for at least three different antibiotics. Meng *et al.* (1998) mentioned that 24% from *E.coli* isolates were resistant to at least one antibiotic and 19% were resistant to three or more of the antibiotics tested. Recently Orden *et al.* (2000) recorded that 77% of the tested *E.coli* strains were resistant for at least double, 67% could resist at least four, and 32% resist at least eight antibiotics.

In the present investigation, the tested *Staph.aureus* strains were multidrug resistant (4% were single, 12% were double, 40% were triple, 40% were quadruple and 4% were sixtuple antibiotic resistant) as shown in Table (5).Gentilini *et al.* (2000) recorded that 64% of the tested *Staph.aureus* were multidrug resistance. Abd El-Hafeez (2000) found that 6.17% were triple, 24.69% were quadruple, 28.39% were quintuple, 24.69% were sixtuple and 16.04% were seventuple antibiotic resistant strains. These significant differences in multidrug resistance percentages necessistates searching for the transferable plasmids encoding genes of antibiotic resistance.

Greater use of an antibiotic has resulted in an increase of *E.coli* strains resistant to that antibiotic. Multiple resistance could increase with the use of one antibiotic where resistance to it and to other antibiotics are on the same plasmid (Jakson, 1981).Concerning *E.coil* strains, results recorded in Table (6) and Fig. (1) indicated the presence of plasmids DNA which their content ranged (1-3) plasmid DNA per *E.coli* strain and molecular size ranged (11-53) kilo base (K.b). David *et al.* (1991) found one to eight plasmids whose molecular size varied from 1.54 to 34.67 Kb. Also Sayed *et al.* (2001) reported that the extracted plasmid contents ranged (1-5) plasmids per *E.coli* strain with molecular size ranged (2.5-27) Kb.

Wise *et al.* (1985) recorded that the seriousness of a plasmid presence appears when it is a transferable one. Nazer (1978); Baldini *et al.* (1983) and Wise *et al.* (1985) showed that 26, 97 and 91% of the tested *E.coli* antibiotic resistant were carrying transferable plasmids.

All *Staph. aureus* strains were negative for plasmid profile analysis (Table 6).

The main requirement in terms of food hygiene is to avoid risks resulting from the presence of pathogenic, potentially pathogenic and toxinogenic microorganisms in food (Pazakova *et al.,* 1997). Rather than its devoiding of any microorganisms carry antibiotic resistance genes (plasmids) as these plasmids may be transmitted to other human flora (Wagner and Hahn, 1999).

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Table 1: Incidence of *E.coli* and *Staph.aureus* strains isolated from luncheon.

Microorganisms	No. + Ve / No. Tested	%	Serotypes	
			No.	%
E.coli	15/ 54	27.8	2	13.3
Staph.aureus	25/ 54	46.3	-	-

Table 2: Antibiotic resistance of *E.coli* strains isolated from luncheon (n = 15)

Types of antibiotics	Resistance of tested strains No.	%
Amikacin	4	26.7
Erythromycin	8	53.3
Chloramphenicol	7	46.7
Amoxil	14	93.3
Ampiclox	14	93.3
Nalidexic acid	0	0
Gentamycin	0	0
Oxytetracycline	0	0
Penicillin	15	100
Tobramycin	1	6.7

Table 3: Antibiotic resistance of *Staph.aureus* strains isolated from luncheon (n = 25)

Types of antibiotics	Resistance of tested strains No.	%
Amikacin	2	8
Erythromycin	4	16
Chloramphenicol	1	4
Amoxil	18	72
Ampiclox	24	96
Nalidexic acid	3	12
Gentamycin	2	8
Oxytetracycline	0	0
Penicillin	25	100
Tobramycin	4	16

Table 4: Multidrug resistance of *E.coli* strains isolated from luncheon

Drug resistance	No. of strains	Percentage
Double:	1	6.7
C& P	1	6.7
Triple:	3	20
P, AX & AMX	3	20
Quadruple:	4	26.7
E, P, AX & AMX	2	13.3
C, P, Ax & AMX	1	6.7
AK, P, Ax & AMX	1	6.7
Quintuple:	6	40
AK, E, P, Ax & AMX	2	13.3
E, C, P, Ax & AMX	3	20
Tob, C, P, Ax & AMX	1	6.7
Sixtuple:	1	6.7
AK, E, C, P, AX & AMX	1	6.7

N.B: All *E. coli* tested were resistant at least against double antibiotics.

GM = gentamycin
E = erythromycin
C = chloramephenicol
AMX = amoxil
AK = amikacin
Tob = tobramycin
NA = nalidixic acid
OT = oxytetracycline
AX = ampiclox
P = penicillin

Table 5: Multidrug resistance of *Staph. aureus* strains isolated from luncheon.

Drug resistance	No. of strains	Percentage
Single:	1	4
P	1	4
Double:	3	12
Ax & P	3	12
Triple:	10	40
AMX, AX & P	8	32
AK, AX, & P	1	4
AX, GM & P	1	4
Quadruple:	10	40
AMX, AX, Tob & P	4	16
AMX, AX, NA & P	1	4
AK, AMX, AX & P	1	4
E, AX, NA & P	1	4
E, AMX, AX & P	3	12
Sixtuple:	1	4
C, AMX, AX, NA, GM &P	1	4

All *Staph.aureus* strains tested were resistant at least against single antibiotic

Fig. 1: plasmid profile analysis of *E.coli* and *Staph.aureus* strains isolated from luncheon.

M: Marker

No. 1 to 5 → *E.coli* strains

No. 6 to 10 → *Staph. aureus* strains

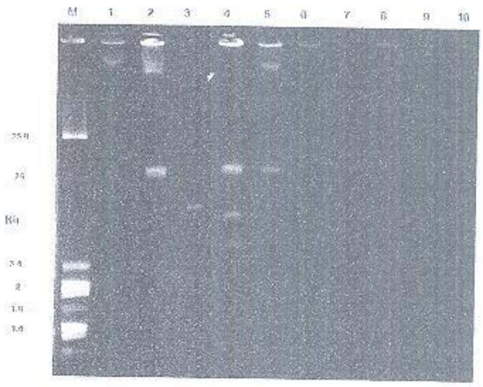

Table 6: Plasmid profile analysis of *E.coli* and *Staph.aureus* strains corresponding to their own multidrug resistance

No.	No. of plasmid DNA	Molecular size of plasmid DNA (K.b)	Multidrug resistance
	E.coli:		
1	1	53	AK, E, P, AX & AMX
2	2	52 & 27	E, C, P, AX & AMX
3	1	20	Tob, C, P, AX & AMX
4	3	29, 18 & 11	AK, E, P, AX, AMX
5	2	53 & 29	Ak, E, C, P, AX & AMX
	Staph.aureus		
6	-	-	C, AMX, AX, NA, GM & P
7	-	-	AMX, AX, NA, & P
8	-	-	AK, AMX, AX & P
9	-	-	E, AMX, Ax & P
10	-	-	E, AMX, AX & P

All *Staph.aureus* strains were negative for plasmid profile analysis

Kb : Kilo base