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HEAITH HAZARD OF ANTIBIOTIC RESISTANCE ENTEROCOCCI ISOLATED FROM ROW CAMEL MILK

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ABSTRACT

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Enterococci spp. were isolated and identified from Camel milk samples in order to evaluate its sensitivity pattern to common use antibiotics. Thirty milk samples were randomlyobtained from local vendors and seller of camel milk from different zones in Upper Egypt (Arment city, Edfoo city and Daraw city). Enterococci were isolated and enumerated using KF Streptcoccal culture media, identified using bio-chemical tests. HemolIysin activity of E.fecalis was done. The study focused on the resistance patterns of the selected hemolytic and non hemolytic E.fecalis strains to 6 antimicrobial active agents (Gentamycin, Nalidixic acid, Ampicillin, Oxytetracclin, Neomycin and Novobiocin). 36.66% of Camel milk samples were positive for enterococcus with a mean count of 1.72 x 10^4± 3.86 x10^3cfu/ ml, 14different strains were isolated, where, E.fecalis corresponded to 64.28 %hemolytic E. fecalis to 7.14%, E.facium 14,28 and eachE. durans and E.hirai 7,14%. All isolates identified showed an important resistance to the antibiotic tested (singly or in combination) Ampicillin, Nalidixica acid and Neomycin showed the high resistance. However, Novobiocin is considered sensitive. The abundance of isolates showing multi drug resistance suggests that the sanitary quality of camel milk should be improved to decrease the incidence of enterococci. Further more. Conventional pasteurization at63C° for 30 min is essential.

Key words: *Health Hazard, Enterococci, Camel milk.*

INTRODICTION

Presently, enterococci take the third place of bacterial pathogens after Staphylococci and Escherichia coli. They are important nosocomial pathogens that cause bacteraemia, endocarditis and other infections (Franz *et al.,* 2003 and Peters *et al.,* 2003). Bacteria resistant to antimicrobial drugs which penetrate into human population with foods of animal origin and rank with direct causative agents of food borne diseases represent a possible source of drug resistance for human pathogenagents (Shryock, 1999). The resistance of enterococci to several available antibiotics is threatening and documented (Gomes *et al.,* 2008).

Nowadays, enterococci are used in the food industry as starter or probiotic cultures (Gomes *et al.,* 2008). The role of enterococci in diseases has raised questions on their safety for use in food (Franz *et al.,* 2003). Moreover, enterococci have a distinctive role as indicator of poor factory sanitation owing to their relatively high resistance to drying, high temperature, detergents or disinfectants. Also theyhave value in assessing both the microbiological safety and quality of food.

For centuries camel milks medicinal properties have been known, it strengthens the immune system as it contains a number of immunoglobulin that are compatible with human ones. Also, there are many protective proteins in camel milk that exert immunologic, bactericidal and viricidal properties (Kappele, 1998).

Camel milk usually consumed in the raw state based on the fact that it has the ability to inhibit growth of pathogenic bacteria (Farah, 1993). Recent studies indicated that camel must be pasteurized because it contains more bacteria than the allowed maximum (Yosefshabo *et al.,* 2005). It has been realized that there is a need to assess the occurrence of enterococci in camel milk and their drug resistance.

MATERIALS and METHODS

Collection of samples:
30 milk samples were randomly obtained from local vendors and seller of camel milk from different zones in upper Egypt (Arment city, Edfoo city and Daraw city). Milksamples transported under refrigeration (4-6 C°) in thermal pox containing ice packs. Milk samples were examined for:

1- Enumeration of enterococci**:**
Ten fold serial dilutions from each sample were prepared according to APHA (1992). Enumeration of enterococci was done using KF streptococcal media according to Deibel and Hartman (1982).
Identification of isolates:
All isolates recovered from the examined samples were identified according to Morrison *et al.* (1987).

2- Hemolytic activity of E.fecalis:
All isolatessuspected to be E. fecalis identified were examined for their ability to produce alpha and or beta hemolys in using blood agar according to Ike *et al.* (1987).

3- Antibiotic susceptibility testing:
Hemolytica and non-hemolytic E. fecalis isolates were tested for antibiotic resistance using the standard disc diffusion method as recorded by NCCLS (1993). Discs containing Ampicillin (10 µg), Nalidixic acid (30 µg), Oxytetracyclin (30 µg), Neomycin (30 µg), Gentamycin (10 µg) and Novobiocin (30 µg) were used. Inhibition zones were interpreted following the guideline tables of the NCCLE (1999). The multiple antibiotics resistance (MAR) index for each isolate was determined, it was defined as a/b, where (a) is the number of antibiotics to which a particular isolate is exposed (Krumperman, 1983).

RESULTS

Table 1: Statistical analytical results of total enterococci isolated from row camel milk samples.

Positive samples		Min.	Max.	Mean	±S.E
No./30	%				
11	36.66	2.50×10^2	1.60×10^5	1.72×10^4	$3.86 X10^3$

Table 2: Frequency distribution of different *enterococci* isolated from row camel milk samples.

Strain no.	Drug resistance pattern	MAR index	Drug sensitivity pattern
E.1(Hemolytic E. Fecalis)	AM, N, NA, CN,T, NV	1.0	-
E.2	AM, N, NA, CN,T	0.80	NV
E.3	AM, N, NA, T	o.62	NV,CN
E.4	AM, N, CN,T	0.62	NV,NA
E.5	AM, N,NA	0.5	NV, CN,T
E.6	AM, NA, CN	0.5	NV, N,T
E.7	N, NA, CN,	0.5	AM,NV, T
E.8	AM, N,	0.32	NA,CN, NV,T
E.9	AM	0.16	NV, CN,NA, T,N

- No of isolated strains 14

Table 3: Antibiotic resistance of hemolytic and non hemolytic E. fecalis isolated from camal milk samples

Range	*E. fecalis*		Hemolytic *E. fecalis*		*E. FacIum*		*E. durans*		*E.hirai*	
	No.	%	No.	%	No.	%	No.	%	No.	%
10^2-10^3	2	14.28			1	7.14	1	7.14		
10^3-10^4	4	29.57	1	7.14	1	7.14			1	7.14
$>10^4$	3	21.42								
Total	9	64.28	1	7.14	2	14.28	1	7.14	1	7.14

- AM Ampicillin 7* CN Gentamycin 5*
- N Neomycin 7* T Oxytetracyclin4*
- NA Nalidixic acid 6*NV Novobiocin1*
- * No. of resistant strains

DISCUSSION

The genus enterococcus is the most controversial group of lactic acid bacteria they have both beneficial and virulence features as it can be used as starter or probiotic cultures and it have been associated with a number of human infections (Foulquie Moreno *et al.,* 2006).

The present study revealed a definite dominance of enterococci in camel milk, it was found in 36.66 % of samples with a mean count of 1.72 x 10^4± 3.86X10^3cfu/ ml (Table 1). Many literature recorded different counts of enterococci in camel milk. Aly and Abo-Al-Yazeed (2003) (3.2x 10^3± 1.4x 10^2) cfu/ml, Benkerroum *et al.* (2003) (2.9x10^5) cfu/ml and Khedid *et al.* (2003) who found unexpected very low numbers in most samples with an average of 20 cfu/ml E. fecalis was clearly the predominant species, it was found in 64.28% isolates, the majority of them (29.57%) lies within the range of 10^3 -10^4, 3samples(21.42) have counts more than 10^4cfu/ml. Besides E. fecalis, other species were identified including hemolytic E. fecalis, E.facium, E. durans and E.hirai in percentage of 7.14, 14.28, 7.14 and 7.14 % respectively (Table 2). E. facalis was the main representative species of samplesin many studies (Benkerroum *et al.,* 2003; Chingwaru *et al.,* 2003; Abriouel *et al.,* 2008; ElShaer; El Ganzoury, 2008). Studies on the incidence of virulence traits among enterococcal strains that E. facalis harbors more of them. E.facium appears to pose a lower risk in food because these strains generally harbor fewer recognized virulence determinants than E. facalis (Franz *et al.,* 2003). Only one sample was identified as hemolytic E. facalis (Table 2). hemolysin plays an important role in enterococcal virulence (Franz 2001). It has been suggested that the absence of hemolytic activity should be a criterion for the selection of starter strains be used in fermented dairy products (Giraffa, 1995).

Antibiotics used in this study represented the major groups of antibiotics used. Almost all of the E. fecalis isolates were resistant to Ampicillin, Neomycin and Nalidixic acid and sensitive to Novobiocin. Strain specific resistance traits were observed for Ampicillin, Neomycin (seven of nine strains). Nalidixic acid (six of nine strains), Gentamycin (five of nine strains), Oxytetracyclin (four of nine strains), Novobiocin (one of nine strains) (Table 3).

Various researchers have noted an increase in the Ampicillin resistance of these species since early 1990 (Suppola *et al.,* 1999, Chingwaru *et al.,* 2003; Abriouel *et al.,* 2008). On other hand, enterococci were sensitive to most drugs as recorded by Peters *et al.* (2003) and Valenzuela *et al.* (2008). So it is necessary to consider the resistance pattern of the enterococci in question before administration any antibiotic (Descheemaeker, 1999). Although the resistance of enterococci is considered atypical and thus possibly acquired in enterococci (Teuber *et al.,* 1999), an increasing number of food born enterococci have developed resistance against various therapeutic antibacterial agents including tetracyclines (Huys *et al.,* 2004) and Gentamts in (Donabedian *et al.,* 2003). Multiple resistances of enterococci to all tested antibiotics were reported by Gelsomino *et al.* (2004). The present study showed that the hemolytic E.fecalis strain (E1) displayedmultiple resistance to all six antibiotics, the other E. fecalis strains showed various degree of multiple resistance. However, one strain (E9) was found resistant to Ampicillin only. Furthermore, high MAR indices of all isolates (0.32- 1.0) except E.9 (o.16) were recorded (Table 3) MAR index of >0.2 are considered indication of high risk source of selective pressure for the development of antibiotic resistance bacteria (Krumperman, 1983).

Camel milk has the ability to inhibit growth of pathogenic bacteria owing to its antibacterial factors (Barbour *et al.,* 1984). Lysosymelactoferrin, lactoperoxidase and immynoglobulin A, G were extracted from camel milk (El sayed, 1992). Lysosome is a milk protein that has bactericidal effect as it capable of degrading the bacterial cell wall and enhancing the activity of the immune antibodies (Barbour *et al.,* 1984). Camel milk lactoperoxidase was bacteriostatic against Gram positive strains and bactericidal against Gram negative cultures while; the immunoglobulin had little effect against bacteria (El sayed, 1992). Despite of these antibacterial properties of camel milk, the incidence and count of enterococci in this study was considered high. This may attributed to the resistance of enterococci strains to these factors. The present study proved high level of multidrug resistance and high MARS indices of the isolated E.fecalis, the resistance of enterococci to the normal antibacterial factors present in camel milk needs more investigations.

As seen, different species of enterococci were isolated from camel milk in varied percentages and counts. Furthermore, high level of multidrug resistant E.fecalis was recorded. The problem was complicated by the fact that camel milkis consumed in the raw state and heat processing is not used as means of preservation. The heat treatments commonly used to cow's milk such as pasteurization and sterilization cause denaturation of the whey protein gel pattern. It was found that pasteurization temperature at 63C° caused no visible change in the whey protein gel patternof camel milk while, stronger heat treatment (80ºC°) resulted in 70-81% denaturation of the whey protein (Farah, 1993).

CONCOLOGEN

The results of the current study indicate that the presence of multidrug resistant enterococci is of concern to public health so, maintenance of proper cleanliness and hygiene during milking to limit the degree of contamination is essential together with pasteurization of 63C°. Moreover, it is necessary to consider the resistance pattern of the enterococci in question before administering any antibiotic.

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المخاطر الصحية من تواجد الأنتيروكوكاي المقاومة للمضادات الحيوية المعزولة من لبن الجمال الخام

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تزايد الاهتمام في الاونه الاخيرة بألبان الجمال لدورها العلاجي وأحتوائها علي حميع العناصر الغذائية ونظرا لان هذه الالبان تستهلك طازجة وقد تكون ملوثة ببعض الميكروبات لذا هدفت الدراسة إلي معرفة مدي تواجد ميكروب الانتيروكوكاي في ألبان الجمال ومدي مقاومتها للمضادات الحيوية الشائعة. جمعت ٣٠عينة من ألبان الجمال وتم عزل الانتيروكوكاي بنسبة ٣٦,٦٦% وكان المتوسط العددي لهم هو ١٠X١.٧٢ ٤ ±٣.٨٦×١٠٣مبكروب/ ملل. تم التعرف علي ١٤ عزلة منها حيث كانت أعلي نسبة تواجد لميكروب ال E. fecalis (٦٤,٢٨%) وتم عزل كل من E. *durans*, E. hirai, Efecalis, hemolytic E. facium, ١٤,٢٨، ٧,١٤، ٧,١٤، ٧,١٤ علي التوالي.تم أجراء إختبار الحساسية علي ٨ عزلات من E. fecalis وعزلة واحدة من hemolytic E. fecalis بأستخدام ٦ أنواع من المضادات الحيوية وقد أظهرت العزلات مقاومة عالية لبعض المضادات الحيوية (مفردة أو مجمعة) من بينهم Ampicillin، nalidixicacid, neomycin قي حين أعتبر Novobiocin هو الآاكثر حساسية لمعظم العزلات. أوضحت الدراسة أن تواجد ميكروب الانتيروكوكاي المقاومة للمضادات الحيوية يشكل خطر علي صحة المستهلك لذا توصي الدراسة بإتباع بعض الخطوات الصحية لضمان خلو اللبن من هذة الميكروبات ونوصي أيضا بأستخدام البسترة البطيئة حفاظا علي صحة المستهلك.