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Full Length Research Paper

Identification and susceptibility of *Klebsiella* and *Enterobacter* spp. isolated from meat products

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Identification and susceptibility to 8 selected antimicrobial agents of *Klebsiella* and *Enterobacter* bacterial species was studied in 15 marketed meat samples (chicken, turkey-hen, beef, sheep, pig, dromedary, ostrich, and fish). The isolates were identified with the API 20E system, resulting in 7 clusters: *Enterobacter aerogenes* (2 isolates), *Enterobacter cloacae* (6), *Enterobacter sakazakii* (3), *Enterobacter spp* (14), *Klebsiella oxytoca* (5), *Klebsiella pneumoniae* (2) and *Klebsiella ornithinolytica* (12). The identities of isolates identified as *Enterobacter* and *Klebsiella spp.* were confirmed by Amplified Ribosomal DNA Analysis (ARDRA), using *Alu*I, *Msp*I, *Rsa*I restriction enzymes. Identification of isolates by ARDRA and API 20E system gave similar results with 90,2 % (44/51) of the collection. Susceptibility to antibiotics was evaluated by the disk method according to French recommendations (CA-SFM). Resistance rates were as follows: Ampicillin (62%), cefalotin (30%), tetracycline (14%), chloramphenicol 2%. aminoglycosides 0% and nalidixic acid 0%. Strains isolated from 'industrially reared animals' showed higher resistance rates than 'naturally reared' ones.

Key words: *Klebsiella*, antibiotic resistance, susceptibility, *Enterobacteriaceae.*

INTRODUCTION

In human medicine, several uses are known for antibiotics such as treatment of infectious disease (example, enteric and pulmonary infections, skin and organ abscessses, as well as mastitis) (Michael, 2001). In veterinary medicine, these or closely related substances are used for pets, farm animals, and animals rose in aquaculture. For farm animals, they are utilized in therapy and prophylaxis. These substances are also considered as factors that increase growth and feed efficiencies of such animals. Moreover, industrial animal farming is associated with large antimicrobial use scale, which led to a high level of animal colonization by antimicrobial-resistant bacteria that can then contaminate food and, in turn, humans (Corpet, 1998) . In the recent years, a substantial increase in antibiotic resistance has been observed, mainly in developing countries (Lester et al., 1990) because of self-medication, the suboptimal quality of bacteria can be transferred to pathogenic species (Doucet et al., 1992; 2001). Enterobacteria are the most frequently described agents in community infections. The antimicrobial drugs, and poor community and patient's hygiene (Wason et al., 2001). Resistance in commensal specimens of this family have been widely exposed to an extensive use of antibiotics in the last 20 years. Consequently, they have developed an increasing resis-tance to the most frequently-used antimicrobial agents. This has been proven in hospital as well as in environ-ment by several multicentric studies (De Moüy, 1991, Péan et al., 1999). Bacteria of the genus *Klebsiella* and *Enterobacter* spp. are among the major causes of noso-comial infections. They often give rise to urinary and respiratory tract infections, and next to *Escherichia coli*, they are the most common cause of Gram-negative bac-teraemia (Podschun and Ullmann, 1998). *Enterobacter* and *Klebsiella* spp. are frequently described in resistant nosocomial infections (Davin-Regli et al., 1996, Martinez-Martinez et al., 2002). The aim of this study was to investigate the role of environmental isolated strains (meat) in the increase of antibiotic resistance.

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MATERIALS AND METHODS

Sample collection and preparation

Food samples (*n* = 15), including ground beef, packed pork sausage, retail packages of beef steak, pork chops, hamburger, fish and products from turkey and chicken origin, were randomly collected from retail stores of five supermarket chains in Tunis and from naturally-raised animals (at a farm in Sidi-Thabet, Tunis, Tunisia). Sampling visits were made one day per week for 6 months (September 2005 to February 2006). Eight prepackaged raw meat products (two of each meat type) were randomly selected and transported on ice to the laboratory. Each sample was aseptically removed and placed in a plastic bag that contained 200 to 500 ml of buffered peptone (Difco Laboratories, Detroit, Mich.), depending on the sample size. The bag was shaken manually for 3 min and left on ice for 20 min.

Bacterial isolation

The collected meat samples were analyzed as follows: 11 g of each sample was suspended in sterile peptone then selenite broth at 37°C for 24 h. According to the standard dilution method, 1 ml from each concentration was plated onto Hektoen and MacConkey agar plates and incubated at 37°C for 24 h. Colonies suspected *Enterobacter* and *Klebsiella* spp. (on the basis of colony size and morphology) were selected for identification and further studies. A total of 51 *Enterobacter* and *Klebsiella* spp. isolates were recovered from the 15 food samples and subjected to further investigations (Table 1).

Biotyping

Biochemical identification was determined by the API 20E system (Api bioMerieux) according to the manufacturer's instructions.

Chromosomal DNA extraction

Chromosomal DNA extraction was carried out as described by Chen and Kuo (Birnboim and Doly, 1979, Chen and Kuo, 1993). After an overnight culture at 37°C on MacConkey agar medium, colonies were suspended in 1.5 ml of Luria broth medium and incubated at 37°C for 24 h. Then, centrifuged (12,000 tpm during 3min at 4°C) and the supernatant were suspended into 200 µl of extraction buffer (Tris acetate [pH 7.8]; 40 mM, sodium acetate; 20 mM, EDTA [pH 8]; 1 mM and SDS 1%). The cell detri-tus was removed by a supply of 66 µl NaCl 5 M and a centrifugation at 12,000 tpm for 10 min at 4°C. The supernatant was later treated with an equal volume of phenol-chloroform (1:1) . The aqueous phase was discarded by 2.5 volumes of frozen absolute ethyl alcohol and two washings in ethyl alcohol 70% (v/v). DNA was purified by TE ((Tris-HCl [pH 7.4]; 10 mM, EDTA [pH 8]; 1 mM) and stored at - 20°C.

Amplification of 16S RNA gene

Amplification of 16S rDNA gene was carried out with universal primers S - D - Bact - 0008 - a - S - 20 and S - D - Bact - 1495 - a - S - 20 . Each reaction tube contained 0.5 µM of each primer, 50 mM Tris -HCl (pH 9.0), 50 mM KCl, 7 mM $MgCl_2$, 2 µl of purified DNA, 100 mM deoxynucleoside triphosphate, and 0.5 U of *Taq* polymerase (ABI, Milano, Italy) and was adjusted to a total volume of 50 µl. Samples were amplified in a Biomed model 60 thermal cycler (Biorad) programmed for a first cycle of denaturation (10 min at 94°C). The 35 subsequent cycles of amplification consisted of denaturation for 1 min at 94°C, annealing for 1 min at 40°C, and extension for 1 min at 72°C, with a final extension step of 10 min at 72°C. The products were electrophoresed on a 1.5% agarose gel and viewed by ethidium bromide staining.

Enzymatic digestion of 16S DNA

For ARDRA analyses (Amplified rDNA Restriction Analysis), the final reaction was carried out in a final volume of 10 µl. 16 S rDNA products were digested in an overnight mixture reaction in the presence of 1 µl of the appropriate buffer, 1 µl of the amplified DNA and 8 µl of restriction enzymes (5 - 10 U / µl): *Alu*I, *Msp*I and *Rsa*I, and resolved on 6% polyacrylamide gel.

Susceptibility testing

Antibiotic susceptibilities were determined by the procedure of standard agar dilution (National Committee for Clinical Laboratory Standards, 1999) on Mueller-Hinton agar. The following antimicrobial agents were tested: Ampicillin, tetracycline, cefalotin, neomycin, kanamycin, gentamicin, nalidixic acid and chloramphenicol. Results were interpreted according to standards and recommendations of the CA-SFM (Comité de l'Antibiogramme de la Société Française de Microbiologie).

Data analysis

Band profiles of electrophoresis gels were analysed using Gel pro software (for Windows, N/T version 3.1, 95). MVSP software was used to estimate levels of similarity between strains based on ARDRA profiles (version 3.13I, Kovach, on 1995).

RESULTS

Isolation and biochemical characterization of strains

On the basis of morphological criteria, 51 isolates were selected from 15 meat samples from different origins (Table 1). There were no isolates able to be grown from fish samples. Biochemical identification in all isolates was performed by using the API 20E system; additional tests (motility, catalase) were performed by conventional methods (Table 2). According to the morphological and biochemical characterizations, the isolated strains were classified into 9 groups related to genera: *Enterobacter* spp., *Klebsiella* spp, and *Escherichia coli* (Table 3).

Molecular identification

A fragment of about 1500 bp was amplified from the 51 isolated strains using the universal primers: S-D-BACT-0008-A-S- 20 and S-D-BACT-1495- A-A-20 (Wheeler et al., 1996) (Figure 1) . ARDRA is a restriction fragment length polymorphism pattern of polymerase chain reaction amplified 16S rDNA gene (Heyndrickx et al., 1996). We applied ARDRA to rDNA 16S gene of isolated strains using *Rsa*I, *Alu*I, and *Msp*I enzymes. The products of digestion were resolved on 6% polyacrylamide gel (Figures 2, 3 and 4). The bands acquired by the three restric-

Table 1. Origin of strain.

Meat type	Origin	Number of isolates
Lamb's meat	Reared animals	1
Meat of ostrich	Carrefour*	1
Meat of turkey	Mliha*	1
Sausage of Frankfort	Mazreaa*	3
Chicken	Mliha*	5
Chicken	Mazreaa*	6
Scallop of turkey	Mazreaa*	6
Chicken	Pinda*	2
Meat of pigs	Geant*	2
Meat of turkey	Carrefour*	6
Meat of pigs	Carrefour*	3
Meat of sheep	Reared animals	6
Meat of dromedary	Carrefour*	4
Sheep	Reared animals	5
Fish	Sea bream	0

(*) Meat supermarket

Table 2. Biochemical reaction profiles in the API 20 E test.

Strains	Catalase	Motility	ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
Group A	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+
Group B	-	-	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+
Group C	-	-	+	+	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+
Group D	-	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-	+	+	+	+	+	+
Group E	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	-	-	+	-	-	-	+
Group F	-	-	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Group G	-	+	+	+	+	+	+	-	+	-	-	+	-	+	-	+	+	+	+	+	+	+
Group H	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+		
Group I	-	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+

ONPG: o-nitrophenyl-β-galactosidase ADH: Argininedehydrolase LDC: Lysinedecarboxylase ODC: Ornithinedecarboxylase CIT: Citrate H2S: Sulfure d'hydrogène URE: Urease TDA: Tryptophanedeaminase IND: Indole VP: Voges-Proskauer GEL: Gelatinase Fermentation of sugar GLU: Glucose MAN: Mannitol INO: Inositol SOR: Sorbitol RHA: Rhamnose SAC: Saccharose MEL: Melibiose AMY: Amygdalin ARA: Arabinose. Note: Positive reactions are indicated with a "+" sign, negative ones with a "-".

Table 3. Clustering of strains according to API 20 E test.

Group	Genus	species
Group A	*Enterobacter*	*Enterobacter aerogenes*
Group D	*Enterobacter*	*Enterobacter cloacae*
Group E	*Enterobacter*	*Enterobacter sakazakii*
Group F	*Klebsiella*	*Klebsiella oxytoca*
Group G	*Escherichia*	*E.coli*
Group B	*Klebsiella*	*Klebsiella ornithinolytica*
Group H	*Enterobacter*	*Enterobacter spp.*
Group C	*Klebsiella*	*Klebsiella pneumoniae*
Group I	*Enterobacter*	*Enterobacter spp.*

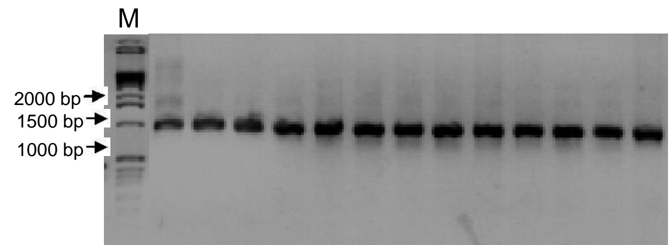


Figure 1. Amplification of rDNA 16S gene by polymerase chain reaction and resolved on 1.5% polyacrylamide gel. M denote the 1 bk DNA ladder.

restriction enzymes were treated by Gel - pro software which generates a matrix containing binary data. The clustering of strains was performed according to UPGMA method using MVSP software. Based on their ARDRA profiles a dendrogramme exhibiting 12 groups with 51 strains was obtained (Figure 5) . Compared with accomplished biochemical identification, seven ARDRA groups (90.19% of strains)

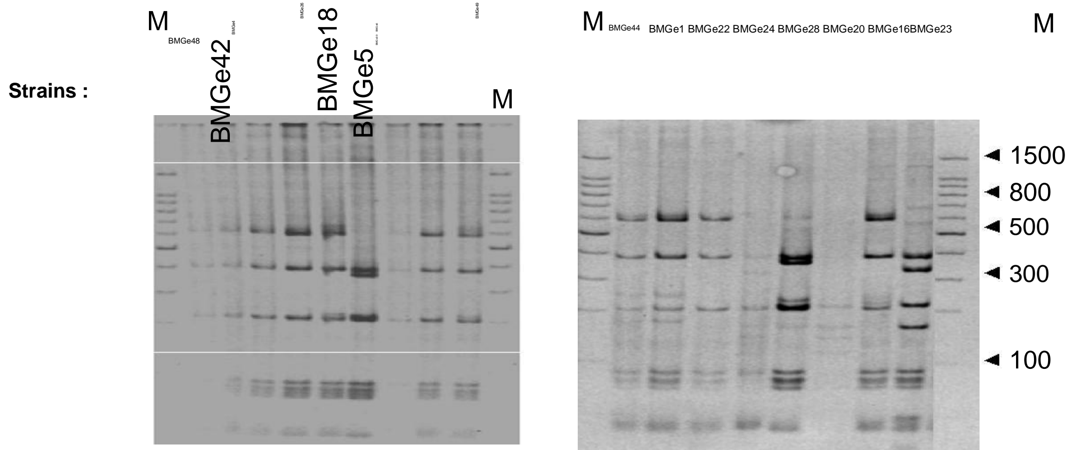


Figure 2. Restriction fragment length polymorphism patterns of polymerase chain reaction amplified 16S rDNA gene digested by the *Alu*I enzyme and resolved on 6% polyacrylamide gel. M denote the 100 bp DNA ladder.

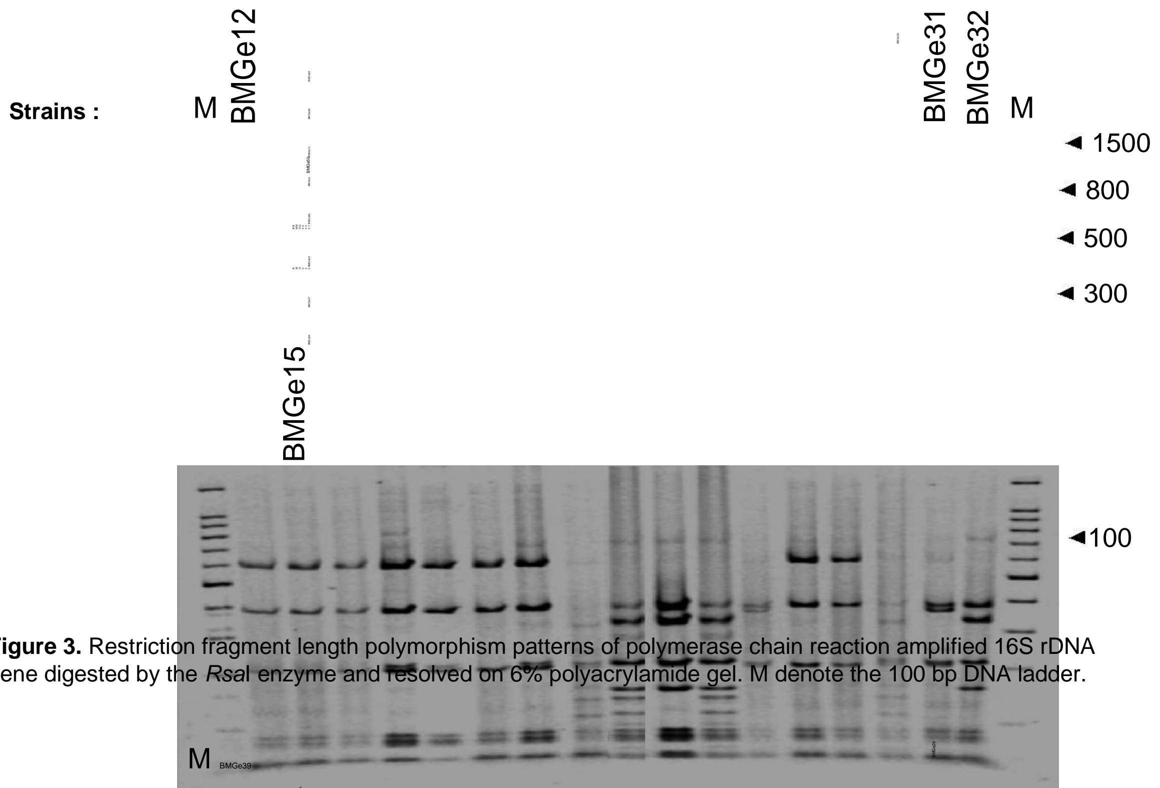
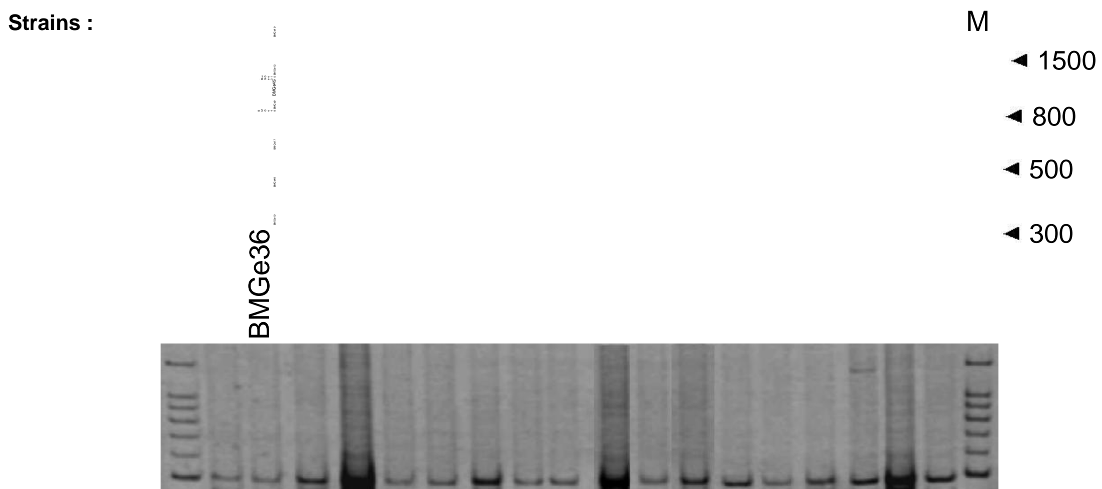


Figure 3. Restriction fragment length polymorphism patterns of polymerase chain reaction amplified 16S rDNA gene digested by the *Rsa*I enzyme and resolved on 6% polyacrylamide gel. M denote the 100 bp DNA ladder.



◄ 100

Figure 4. Restriction fragment length polymorphism patterns of polymerase chain reaction amplified 16S rDNA gene digested by the *Msp*I enzyme and resolved on 6% polyacrylamide gel. M denote the 100 bp DNA ladder.

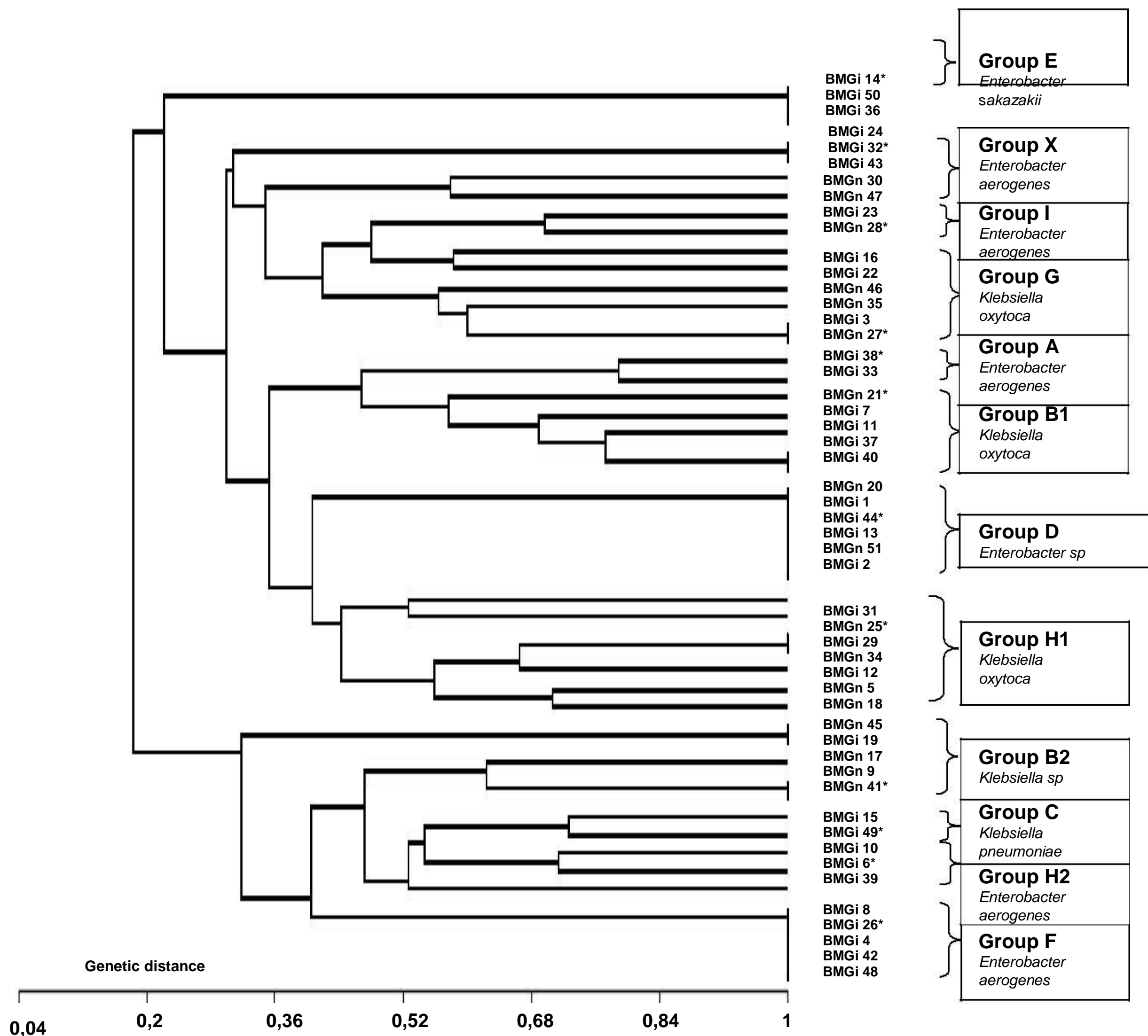


Figure 5. Dendrogram of the percent genetic similarity estimated by comparison of ARDRA profiles *: strains select ed for the DNA s equenci ng BMGi: strai n from industri al ori gi n BMGn: strain from nat ural origin

were found correlating with the Api 20E system grouping (Table 4), respectively: A, C, D, E, F, G and I.

Identification by 16S rDNA sequencing

One strain of every ARDRA group was chosen for this identification. The sequencing of a 700 bp fragment was realized using the primer L-D- Bact-0035-a-A-15. These sequences were aligned by blast software (http:// www.ncbi.nlm.nih.gov / BLAST/) and results are mentioned in Table .

Susceptibility of strains to antimicrobial agents

The analysis of strain resistance to antimicrobial agents shows that gentamicin, kanamicin, neomicin and nalidixic acid inhibited the growth of all isolates. Other antibiotics show differential susceptibility: Chloramphenicol to which only 2% of isolates were resistant, tetracycline (14%), cefalotin (27%) and ampicillin (63%). Calculated Pearson coefficients (r = 0.999) revealed a positive correlation between antibiotic resistance and the origin of strains (industrial origin versus natural origin) (Figure 6). This study suggests that strains isolated from 'industrially-reared animals' (chicken, ostrich, turkey, and pork) showed higher resistance rates than those isolated from 'naturally-reared' ones for all antibiotics. This difference was explained by the use of antimicrobial agents in veterinary medicine and food animal production (Figure 6).

The comparison between ARDRA groups and antibiotic phenotype shows a perfect correlation in 5 groups: A, E, F, H2 which belong to *Enterobacter aerogenes* spe-

Table 4. Correlation between ARDRA, Api20E groups and phenotype

Api 20^E group	ARDRA group	Number of strains	Phenotype
A	A	2	AM^r TE^s RA^r CF^s
B	B1	5	AM^r TE^s RA^r CF^s
	B2	5	variable phenotype
C	C	2	variable phenotype
D	D	6	variable phenotype
E	E	3	AM^r TE^s RA^r CF^s
F	F	5	AM^r TE^s RA^r CF^s
G	G	6	variable phenotype
H	H1	7	variable phenotype
	H2	3	AM^r TE^r RA^r CF^r
I	I	2	variable phenotype
Y	X	5	variable phenotype

-Groups found by ARDRA and api 20 E grouping are written in capital bold letters. -
Groups whose strains has a unique phenotype are written in capital underlined letters.

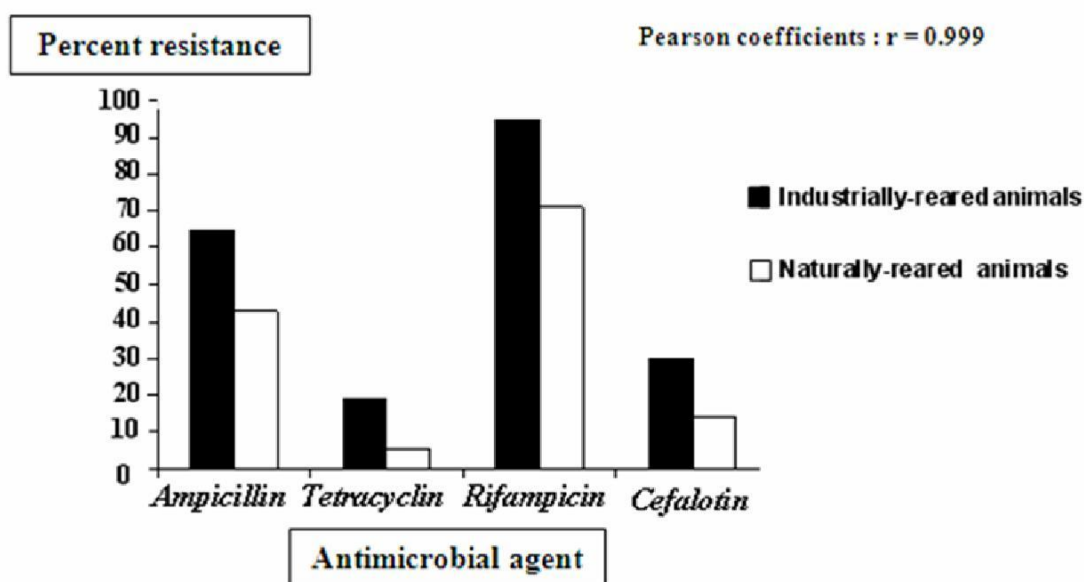


Figure 6. Comparison between the antibiotic resistance of natural origin and those of industrial origin.

cies and B1 which belong to *Klebsiella oxytoca* species.

DISCUSSION

Morphological and biochemical identification showed a higher rate of strains of *Enterobacter* spp. (49.01%) compared to *Klebsiella* spp. (33.33%) . This result agrees with studies of Michael et al. (Michael et al., 1981) focused on the biochemical identification of a collection of 2,200 isolates belonging to the family *Enterobacteriacea* isolated from various meat samples. Table 1 show that strains were predominantly isolated from the samples of meat of fowl, sheep, while in the samples of fish no strains were isolated. The Api 20 E is a system conceived for the identification of the bacteria of the family of *Enterobacteriacea*. The reliability of this technology is estimated at 79% (Caroline et al., 2003) and employing additional tests (oxidase, agility, reduction of nitrate) 95.2% of strains are correctly identified.

Table 5 shows the abundance of *Enterobacter aerogenes* and *Klebsiella oxytoca* species. They represent respectively 31 and 35% of the isolated stocks; the rest is divided in *E. sakazakii* (3 strains), *Enterobacter spp.* (6 strains), *Klebsiella pneumoniae* (2 strains) and *Klebsiella spp.* (5 strains). Studies of Michael and al. (1) confirm that *Klebsiella* and *Enterobacter* bacteria are phylogenetically closely related what can explain the difference between biochemical and molecular identification in the current study. The amplification of 16S-23S intergenic spaces with primers targeting conserved regions could

Table 5. Identification of strains according to 16S rDNA gene sequencing.

ARDRA Group	Number of strains	Strains chosen for sequencing	Genus and species
E	3	BMGe14	*Enterobacter aerogenes*
X	5	BMGe32	*Enterobacter aerogenes*
I	2	BMGe28	*Enterobacter sakazakii*
G	6	BMGe27	*Klebsiella oxytoca*
A	2	BMGe38	*Enterobacter aerogenes*
B1	5	BMGe21	*Klebsiella oxytoca*
D	6	BMGe44	*Enterobacter sp*
H1	7	BMGe25	*Klebsiella oxytoca*
C	2	BMGe49	*Klebsiella pneumoniae*
H2	3	BMGe6	*Enterobacter aerogenes*
F	5	BMGe26	*Enterobacter aerogenes*
B2	5	BMGe41	*Klebsiella sp*

from animal to human via feeding has been well shown by (Perrier et al., 1998; Peter et al., 2005).

Protein and energy undernutrition in many developing countries remains an enormous problem. The increased production and consumption of animal products (especially meats) have been seen as a solution for protein undernutrition. Many governments have adopted policies to promote “industrialized meat” production by administration of in-feed antibiotics. Critics of the routine use of antibiotic additives in industrialized meat production have pointed mainly to the risk of spread of antibiotic-resistant bacteria to people via the food chain and wastewater (Falkow and Kennedy, 2001, Ferber, 2000). In opposition to this position, others have argued that continuous use of in-feed antibiotics is essential for the economic viability of industrialized meat production and that, in turn, Industrialized meat production is necessary to solve protein undernutrition in developing countries. There have even been suggestions that starvation would result without this antibiotic use (Peter et al., 2005).

Conclusion

In conclusion the work in the animal business companies is a risk factor for the acquisition of bacteria resistant to antibiotics. The use of antibiotics as growth promoting factors contributes to the increase of antibiotic resistance, and the subsequent transmission of these resistances in the environment. In our study 'industrially-reared animals' showed higher antibiotic resistance than those from natural origin.

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REFERENCES

Birnboim H, Doly J (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7:1513–1523.

Caroline M, Ohara S, Michael J (2003). Evaluation of the Vitek 2 ID-GNB Assay for Identification of Members of the Family Enterobacteriaceae and Other Nonenteric Gram-Negative Bacilli and Comparison with the Vitek GNICard. J. Clin Microbiol. 41: 2096–2101.

Chen W, Kuo T (1993). A simple and rapid method for the preparation of Gram negative bacterial genomic DNA. Nucleic Acids Res. 21: 2260.

Corpet DL (1988). Antibiotic resistance from food. New England J. Med. 18: 1206-1207.

Davin-Regli A, Monnet D, Saux P, Bosi C, Charrel R, Barthelemy A (1996). Molecular epidemiology of *Enterobacter aerogenes* acquisition: one-year prospective study in two intensive care units. J. clin. Microbiol. 34: 1474-1480.

De Moüy D (1991). Fréquence et sensibilité des germes isolés des infections urinaires en pratique de ville de 1986 à 1990. Pathologie Biologie 39: 495-499.

Doucet F, Trieu-cuot P, Andremont A, Courvalin P (1992). Conjugal transfert of plasmid DNA from *Enterococcus faecalis* to *Escherchia coli* in digestive tracts of gnotobiotic mice. Antimicrobial Agents Chemotherapy 36: 502-504.

Doucet F, Trieu-cuot P, Andremont A, Courvalin P (2001). Inductible transfert of conjugative transposon Tn1545 from Enterococcus faecalis to Listeria monocytogenes in the digestive tracts of gonobiotic mice. Antimicrobial Agents Chemotherapy 35: 185-187.

Falkow S, Kennedy D (2001). Antibiotics animals and people again! Science 291: 397.

Ferber D (2000). Superbugs on the hoof?. Science 288:792–4.

Hélène A, Antoine A, Michel L, Didier D (2001). Résistance aux antibiotiques des bactéries commensales chez les éleveurs de porcs. BMC Microbiology 12 : 39.50.

Heyndrickx M, Vandemeulebroecke K, Hoste B, Janssen, P., Kersters K, de Vos P (1996). Reclassification of Paenibacillus (formerly *Bacillus) pulvifaciens* (Nakamura 1984). Int. J. Syst. Bacteriol. 46: 270-9.

Jensen M, Webster J, Straus N (1993). Rapid identification of bacteria on the basis of polymerase chain reaction-amplified ribosomal DNA spacer polymorphisms. Appl. Environ. Microbiol. 59: 945–952.

Lester S, Delpilar M, Wang F, Perez S, O’briens T (1990). The carriage of *Escherichia coli* resistants to antimicrobial agents by healthy children in Boston, Caracas and Venezuela, and in Qin Pu, China. New Eng. J. Med. 9:285-325.

Martinez-Martinez L, Pascual A, Conejo M, Garcia I, Joyanes P, Domenech-Sanchez A (2002). Energy-dependent accumulation of norfloxacin and porin expression in clinical isolates of *Klebsiella pneumoniae* and relationship to extended-spectrum -lactamase production. Antimicrobial Agents Chemotherapy 46: 3926–3932.

Michael E, Stiles S, Lai-king N (1981). Biochemical characteristics and identification of enterobacteriaceae isolated from meats. Appl. Environ. Microbiol. 50 : 639-645.

Michael T (2001). Veterinary use and antibiotic resistance. Current Opinion in Microbiology 4: 493–499.

Molbak K, Baggesen D, Aaerestrup F, Frydendahl K (1999). An outbreak of multidrug-resistant, quinolone-resistant Salmonella enterica serotype typhimurium DT N. New Eng. J. Med. 19: 1420-1425.

National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing: ninth information supplements (M100-S9). National Committee for Clinical Laboratory Standards, Wayne. P. 1999.

Péan Y, Goldstein F, Guerrier M, De Bels, F (1999). Les membres de Vigil'Roc (Ville et Hôpital). Sensibilité aux -lactamines des bactéries isolées en ville et à l'hôpital au cours d'une enquête multicentrique française. Antibiotiques 1: 165-70.

Perrier C, Courrier P, Breaard J, Vignot J, Masseront T, (1998). Entérocoques résistants aux glycopeptides dans les viandes. Bulletin Epidemiological Hebdo 12: 37.20

Peter C, Henrik C, Peter B, Colin D (2005). The Routine use of antibiotics to promote animal growth does little to benefit protein undernutrition in the developing world. Clinical Infectious Diseases 41: 1007–1013.

Podschun R, Ullmann U, (1998). *Klebsiella spp.* as nosocomial pathogens: epidemiology, taxonomy, typing methods and pathogenicity factors. Clin. Microbiol. Rev. 11: 589 ± 603.

Wason J, Marshall B, Pokhrel B, Kafle K, Levy S (2001). Carriage of antibiotic-resistant fecal bacteria in Nepal reflects proximity to Kathmandu. J. Infect. Dis. 9(184): 1163.

Wheeler A, Oerther D, Larsen N, Stahl D, Raskin L (1996). The oligonucleotide probe database. Appl. Environ. Microbiol. 62: 3557-3559.