Examination of Multidrug-resistant *Enterobacteriaceae* Isolated from Salted Cattle Hides and Sheep Skins

by
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Abstract

Antibiotic resistance profiles in *Enterobacteriaceae* isolated from salted cattle hides and sheep skin samples were examined in this study. Antibiotic resistance profiles of 27 cattle hide and 28 sheep skin isolates, obtained from five salted cattle hide and five skin samples originating in different countries such as Dubai, Turkey, Israel, Australia, Lebanon, U.S.A. and South Africa, were examined by disc diffusion susceptibility method using 24 different antimicrobial agents. Seventy percent of the salted hide isolates and sixty-eight percent of the salted sheep skin isolates exhibited resistance to three or more of 24 antimicrobial agents used. Less than 50% of the isolates was resistant to tobramycin (13%), cephalothin (16%), tetracycline (16%), amoxycillin-clavulanate (25%), ampicillin-sulbactam (29%), piperacillin-tazobactam (38%), cefoxitin (20%), ceftriaxone (45%), ceftazidime (33%), cefuroxime sodium (45%), trimethoprim-sulfamethoxazole (25%), ampicillin (45%), chloramphenicol (35%) and nalidixic acid (42%). Although 71% of isolates exhibited resistance to aztreonam, all isolates were susceptible to norfloxacin. Resistance to amikacin (5%), streptomycin (9%), kanamycin (9%), gentamicin (5%), imipenem (4%), meropenem (2%), ciprofloxacin (5%) and ofloxacin (2%) was not very common among the isolates. Our research results showed that multidrug-resistant *Enterobacteriaceae* were common on both salted cattle hide and sheep skin samples. Therefore, we suggest effective antibacterial applications during salt curing of hides and skins to eradicate these multidrug-resistant bacteria in the leather industry.

Introduction

Antibiotics are mostly used in animals for disease control, prevention and treatment, growth promotion and to decrease waste production.[1,2] Although antibiotics are used to kill or inhibit the growth of pathogenic bacteria in humans and animals, some bacteria can become resistant to commonly used antibiotics. Researchers reported that members of the family *Enterobacteriaceae* and the genera *Pasteurella* and *Staphylococcus* easily can become resistant to certain antibiotics.[2] It has been known that genes encoding antibiotic resistance are actually found in every microorganism that produces antibiotics. Genetic basis of this resistance may be chromosomal, plasmid or both.[3]Antibiotic resistance genes may be transmitted via mobile genetic elements, transposons and integrons to other microorganisms.[4] Inactivation of antibiotic, development of resistant biochemical pathway, reduced permeability, the multidrug efflux systems which pump antibiotics out of bacterial cell, alteration of target and quorum sensing are important resistance mechanisms against various antibiotics.[3,5,6] These resistance mechanisms may cause multidrug resistance in different bacterial species.[3,6]

According to the WHO's global report on antibiotic resistance, antimicrobial resistance against disease-causing microorganisms has increased dramatically in almost every country and across numerous sectors.[7] Researchers reported that antibiotic resistance was the most important threat to the successful treatment of microbial diseases.[7] Over the past ten years, the development of antimicrobial resistance in both humans and animals has been increasingly reported.[7,8]

Overuse and misuse of antibiotics in humans, animals and agriculture has been thought to promote development of antibiotic-resistant bacteria.[3,7] Therefore, common use of antibiotics in humans and animals has caused the increased prevalence of antibiotic resistance among microorganisms.[1,3,9-11]

Antimicrobial agents have been used mostly to treat enteritis, pulmonary infections, organ abscesses, mastitis and foot infections in animals.[12,13]

Cephalosporins and β-lactams (ceftiofur, penicillin G, amoxicillin), macrolides and lincosamides (tylosin, tilmicosin, tulathromycin, lincomycin), aminoglycosides (spectinomycin,

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streptomycin, gentamicin, neomycin), fluoroquinolones (enrofloxacin, danofloxacin, ciprofloxacin, difloxacin, marbofloxacin, orbifloxacin), tetracyclines (tetracycline, oxytetracycline, chlortetracycline), sulfonamides (sulfacytine, sulfisoxazole, sulfamethizole, sulfadiazine, sulfamethoxazole, sulfapyridine), polypeptides (bacitracin), streptogramins (virginiamycin), pleuromultilin (tiamulin), phenicols (florfenicol), bambermycins (bambermycin), quinoxalines (carbadox), aminocoumarins (novobiocin, clorobiocin, coumermycin A1) have been reported as antimicrobials used in animals or humans.[14-19]

Sawant et al. (2007) examined feces of healthy lactating dairy cattle to evaluate antibiotic resistance of Gram-negative enteric bacteria against ampicillin, florfenicol, enrofloxacin, spectinomycin and tetracycline. Ampicillin and tetracycline resistant Gram-negative enteric bacteria were isolated from 72 of 211 cows on 17 dairy herds and 89 of 212 cows on 19 dairy herds, respectively. Although florfenicol-resistant and spectinomycin-resistant Gram-negative enteric bacteria were respectively isolated from 18 of 213 cows on 9 dairy herds and 10 of 213 cows on 6 dairy herds, enrofloxacin resistant Gram-negative enteric bacteria were not isolated from cows.[11]

Investigators stated that intestines of humans and animals are the main reservoir of antibiotic-resistant microorganisms. It was also emphasized that water, food and environment may be contaminated with multidrug-resistant Gram-negative enteric bacteria originate from humans and animals.[4] Due to high prevalence of multidrug-resistant Gram-negative *Enterobacteriaceae*, it is not easy to combat diseases caused by enteric bacteria in recent years.[4,20,21]

Although there are several studies that examine antibiotic resistance of *Enterobacteriaceae* in animals and humans, there is no detailed study about antibiotic resistance patterns in *Enterobacteriaceae* isolated from salted cattle hides and sheep skins.[4,11,20-29] Hence, the goal of this experiment was to study *Enterobacteriaceae* isolated from salted cattle hides and sheep skins in order to gauge their resistance profiles against commonly used antibiotics.

Experimental

Test Microorganisms

One strain of *Citrobacter koseri* (SS5), one strain of *Proteus penneri* (SS3), one strain of *Serratia ficaria* (SS1), two strains of *Serratia marcescens* (SS3, SS4), three strains of *Cedecea lapagei* (HS2, HS3, HS4), one strain of *Enterobacter sakazakii* (HS3), two strains of *Ewingella americana* (HS1, HS4), one strain of *Raoultella ornithinolytica* (HS5), four strains of *Enterobacter cloacae* (HS1, HS2, SS2, SS3), six strains of *Escherichia coli* (HS1, HS5, SS2, SS3, SS4, SS5), two strains of *Escherichia vulneris* (HS1, SS2), two strains of *Klebsiella pneumoniaea* ssp. *ozaenae* (HS1, SS5), three strains of *Klebsiella oxytoca* (HS4, HS5, SS3), four strains of *Proteus vulgaris* (HS2, HS4, SS3, SS5), two strains of *Raoultella planticola* (HS5, SS1), two strains of *Serratia odorifera* (HS1, SS1), two strains of *Serratia liquefaciens* (HS3, SS1), four strains of *Serratia plymuthica* (HS3, SS2, SS4, SS5), seven strains of *Serratia rubidaea* (HS1, HS2, HS3, SS2, SS3, SS4, SS5), and five strains of *Yersinia enterocolitica* (HS1, HS2, HS4, SS3, SS5), isolated from salted hide and skin samples in our previous study, were used as test strains in this study.[30] The cattle hides (HS1-HS5) were salt-cured in Dubai, Turkey and Israel; the sheep skin samples (SS1-SS5) were salt-cured in Australia, Lebanon, U.S.A. and South Africa. These strains were identified using the API 20E test kits (Biomèrieux, France) in the study of Ulusoy and Birbir (2015).

Antibiotic Susceptibility Test

Each of the 55 isolates obtained from the samples was grown on Mueller Hinton agar at 37°C for 24 hours. After incubation, each isolate was inoculated into Mueller Hinton Broth and incubated at 37°C for 24 hours. Then, each of the isolates was suspended in sterile saline solution (0.85% NaCl) to adjust the density of the bacterial cultures to McFarland Turbidity Standard No 0.5. Antibiotic susceptibility of test isolates was examined by disc diffusion susceptibility method.[31,32] The antibiotic tests of the isolates were carried out applying a bacterial inoculum of approximately $1x10^8$ CFU/mL to the surface of Mueller Hinton agar plate. A total of 24 different antimicrobial agents belonging to ten antimicrobial categories such as aminoglycosides, β-lactam, β-lactam/β-lactamase inhibitor combinations, carbapenems, cephems including cephalosporins 1st, 2nd and 3rd generations, quinolones, fluoroquinolones, folate pathway inhibitors, monobactams, phenicols and tetracyclines were used in this study (Table I). These antimicrobial agents were placed on the inoculated agar surface and the plates were incubated for 24 hours at 37°C. Then, the zones of growth inhibition around each of the antibiotic discs were measured and evaluated using the criteria described by the Clinical and Laboratory Standards Institute[31] and European Committee on Antimicrobial Susceptibiliy Testing.[32] All antibiotic discs were obtained from Oxoid (Basingstoke, Hants, UK).

Results and Discussion

Our study results obtained from hide and skin samples preserved in different countries showed that each sample contained multidrug-resistant members of the family *Enterobacteriaceae*. The percentage of multidrug resistance (≥ 3) of the hide isolates (70%) was found similar to that of the skin isolates (68%) (Tables I and II).

Although neither monobactams nor carbapenems are used in food-producing animals in European Union countries,[14] the highest antimicrobial resistance of the test isolates (39 isolates, representing 14 different bacterial species) was detected against the monobactam (aztreonam) and resistance to carbapenems (imipenem and meropenem) was detected at low level in our isolates.

Aminoglycosides (amikacin, streptomycin, tobramycin, kanamycin, gentamicin); β-lactam, β-lactam/β-lactamase inhibitor combinations (ampicillin, amoxycillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam); carbapenems (imipenem, meropenem); cephalosporins 3rd generations (ceftriaxone, ceftazidime); quinolones and fluoroquinolones (nalidixic acid, ciprofloxacin, norfloxacin, ofloxacin); and monobactams (aztreonam) used in our study, were reported as "critically important antimicrobials" in the 3rd revision of the WHO list of critically important antimicrobials for human medicine.[17] While phenicols (chloramphenicol), cephalosporins 1st and 2nd generations (cephalothin, cefuroxime sodium, cefoxitin), and folate pathway inhibitors (trimethoprim-sulfamethoxazole) used in our study were stated as "highly

Table I

Antibiotic resistance profiles of 55 isolates belonging to family *Enterobacteriaceae*.

Antimicrobial Categories and Agents	C.lapagei	E.sakazakii	E.americana	R.ornithinolytica	C.koseri	P.penneri	S. ficaria	S.marcescens	E.cloacae	E.coli	E.vulneris	K.pneumoniaea ssp. ozaenae	K.oxytoca	P.vulgaris	R.planticola	S.odorifera	S.liquefaciens	S.plymuthica	S.rubidaea	Y.enterocolitica
Hide isolate numbers	3	1	2	1	-	-	-	-	2	2	1	1	2	2	1	1	1	1	3	3
Sheep isolate numbers	-	-	-	-	1	1	1	2	2	4	1	1	1	2	1	1	1	3	4	2
Aminoglycosides																				
Amikacin	S[a]	S	S	S	S	S	R	S	S	S	S	R	S	S	S	S	S	S	S	S
Streptomycin	S	R	S	S	S	R	R	R	S	S	S	S	I	I	I	S	S	S	S	S
Tobramycin	S	S	S	S	R	S	R	S	S	S	I	S	S	S	S	S	S	S	S	R
Kanamycin	S	S	S	S	S	I	R	S	S	S	I	R	S	I	S	R	S	S	I	I
Gentamicin	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S	S
β-lactam, β-lactam/ β-lactamase inhibitor combinations																				
Ampicillin	S	R	I	R	I	I	R	S	R	S	S	R	S	S	S	R	R	I	R	R
Amoxicillin-clavulanate	S	R	S	S	S	S	R	S	R	S	S	R	S	S	S	R	S	R	S	S
Ampicillin-sulbactam	S	R	S	S	S	S	S	R	S	S	S	R	S	S	S	R	R	S	R	I
Piperacillin-tazobactam	R[b]	R	S	S	R	R	S	S	S	S	S	R	S	R	S	S	R	I	R	S

Table I continued on following page.

Table I continued.

Carbapenems																				
Imipenem	S	I	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S
Meropenem	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
Cephems including cephalosporins I, II, and III																				
Cefoxitin (2nd generation)	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	R	S	S	R	S
Ceftriaxone (3rd generation)	R	R	R	R	S	R	S	S	R	S	S	R	S	S	S	R	S	R	S	R
Ceftazidime (3rd generation)	R	R	R	R	R	R	I	S	R	S	I	S	S	S	I	S	S	I	I	R
Cephalothin (1st generation)	S	R	S	I	R	R	S	R	S	I	I	R	I	S	S	R	S	S	S	I
Cefuroxime sodium (2nd generation)	R	R	I	R	I	R	S	R	R	S	S	R	S	S	I	R	S	R	S	R
Quinolones																				
Nalidixic acid	I[c]	I	I	R	I	I	I	R	R	S	S	R	S	S	R	I	S	I	R	R
Fluoroquinolones																				
Ciprofloxacin	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Norfloxacin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Ofloxacin	S	S	S	S	S	R	S	S	S	S	I	S	S	S	S	S	S	S	S	S
Folate Pathway Inhibitors																				
Trimethoprim-sulfamethoxazole	S	S	S	S	I	R	S	S	R	S	S	R	S	S	S	R	S	S	S	R
Monobactams																				
Aztreonam	R	R	R	R	I	R	R	R	R	S	S	I	S	R	R	S	R	R	R	R
Phenicols																				
Chloramphenicol	R	R	I	I	S	R	I	S	I	S	S	S	S	S	S	R	S	I	R	R
Tetracyclines																				
Tetracycline	S	S	I	S	S	S	R	R	S	S	S	S	S	S	S	R	S	R	S	S
Total numbers of susceptible isolates	16	11	16	16	15	11	11	17	15	23	18	11	22	20	19	11	20	14	15	12

[a]S: Susceptible, [b]R: Resistant, [c]I: Intermediate

important antimicrobials," fluoroquinolones, 3rd and 4th generation cephalosporins and macrolides were reported as "critically important antimicrobials with highest priority."[17] While resistance to trimethoprim-sulfamethoxazole, ciprofloxacin (fluoroquinolones) and tetracycline (tetracyclines) was respectively 25%, 5% and 16%, resistance to ampicillin (β-lactam) was 45% (Tables I and II). Tetracyclines have been known as broad-spectrum antimicrobial class extensively used in animals for many years.[14] Moreover, resistance to cephalosporins including 1st, 2nd and 3rd generations was detected in our isolates (Tables I and II).

Especially fluoroquinolones and 3rd and 4th generation cephalosporins have been used for treatment of invasive Gram-negative bacterial infections in humans.[14] Although 3rd and 4th generation cephalosporins were commonly used in hospital, fluoroquinolones were commonly used in the community (primary care human medicine).[14,17] Forty-five and thirty-three percent of our isolates showed respectively resistance to ceftriaxone and ceftazidime, which are known as 3rd generation cephalosporin, but resistance to ciprofloxacin (fluoroquinolones) was 5% (Tables I and II).

Strains of *Citrobacter koseri* (SS5), *Proteus penneri* (SS3), *Serratia ficaria* (SS1) and *Serratia marcescens* (SS3, SS4), which were isolated only from sheep skins, were found to be resistant to four, ten, ten, and seven antimicrobial agents, respectively. Strain of *C. koseri* was resistant to tobramycin, piperacillin-tazobactam, ceftazidime and cephalothin in our study. Resistance of some *C. koseri* strains, isolated from patients, against ceftazidime, amikacin, piperacillin-tazobactam, imipenem, meropenem and ciprofloxacin was stated by researchers in 2007.[22,33] Intrinsic resistance of *C.koseri* against ampicillin-sulbactam was also demonstrated.[33] In the present study, strain of *Proteus penneri* (SS3) was resistant to streptomycin, piperacillin-tazobactam, ceftriaxone, ceftazidime, cephalothin, cefuroxime sodium, ofloxacin, trimethoprim/sulfamethoxazole, aztreonam and chloramphenicol. *P. penneri* strains were resistant to penicillin G, amoxicillin, cephalosporins (cefaclor, cefazoline, cefuroxime, cefdinir), oxacillin and most macrolides.[23] *P. penneri* strains were susceptible to aminoglycosides, carbapenems, aztreonam, quinolones and sulphamethoxazole.[23] In our study, cephalosporin resistance (ceftriaxone, ceftazidime, cephalothin and cefuroxime sodium) of *P. penneri* isolates was also detected. Other research findings mention intrinsic resistance of *P. penneri,* responsible for healthcare-associated infections, against cefuroxime, ampicillin and tetracycline.[33]

Our investigation found the strain of *Serratia ficaria* (SS1) was resistant to amikacin, streptomycin, tobramycin, kanamycin, gentamicin, amoxycillin-clavulanate, meropenem, aztreonam, ampicillin and tetracycline. *S. ficaria* exhibited remarkable resistance against all aminoglycosides tested (Tables I and II). *S. ficaria* isolated from clinical sample was resistant to ampicillin, amoxicillin-clavulanate potassium, cefotaxime, cephalothin and trimethoprim.[34]

In the present study, strains of *Serratia marcescens* (SS3, SS4) were resistant to streptomycin, ampicillin-sulbactam, cephalothin, cefuroxime sodium, aztreonam, nalidixic acid and tetracycline. Researchers emphasized the intrinsic resistance of *S. marcescens*, responsible for healthcare-associated infections, against cefuroxime, ampicillin, amoxycillin-clavulanate and ampicillin-sulbactam.[33] In another study, *S. marcescens* and *S. liquefaciens* were resistant to tetracycline, amoxycillin-clavulanate, cefuroxime and cefoxitin but susceptible to tobramycin, amikacin, gentamicin, streptomycin, meropenem, imipenem, kanamycin, aztreonam, ceftriaxone, ceftazidime, cephalothin, ciprofloxacin, norfloxacin, ofloxacin and chloramphenicol.[35]

Strains of *Cedecea lapagei* (HS2, HS3, HS4), *Enterobacter sakazakii* (HS3), *Ewingella americana* (HS1, HS4) and *Raoultella ornithinolytica* (HS5), which were isolated only from hide samples, were resistant to seven, eleven, three and six antibiotics, respectively. Strains of *Cedecea lapagei* (HS2, HS3, HS4) were resistant to piperacillin-tazobactam, ceftriaxone, ceftazidime, cefuroxime sodium, ciprofloxacin, aztreonam and chloramphenicol (Tables I and II). In the study of Çekin et al. (2014), *C. lapagei,* isolated from urinary tract infection, was found to be susceptible to amikacin, tobramycin, gentamicin, imipenem, meropenem, cefoxitin, cephalothin, trimethoprim/sulfamethoxazole and ampicillin. Although *C. lapagei* was found to be resistant to piperacillin-tazobactam, ciprofloxacin and aztreonam in the present study, the researchers found susceptible this strain to these antibiotics.[24]

Strain of *Enterobacter sakazakii* (HS3) exhibited resistance to streptomycin, ampicillin, amoxycillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, ceftriaxone, ceftazidime, cephalothin, cefuroxime sodium, aztreonam and chloramphenicol. This strain was resistant to all β-lactam, β-lactam/β-lactamase inhibitor combinations, cephalosporins except cefoxitin, monobactam and phenicol categories tested in this study. *E. sakazakii* infections have been traditionally treated with ampicillin-gentamicin or ampicillin-chloramphenicol.[26]

Strains of *Ewingella americana* (HS1, HS4) were found to be resistant to ceftriaxone, ceftazidime and aztreonam. Our results were consistent with those of other scientists.[27] In the study of Bukhari et al. (2008), *E. americana* isolated from a patient was found to be resistant to ceftriaxone and ceftazidime.

Strain of *Raoultella ornithinolytica* (HS5) exhibited resistance to ceftriaxone, ceftazidime, cefuroxime sodium, aztreonam, ampicillin and nalidixic acid. In the study of Morais et al. (2009),

TABLE II

Multidrug resistance profiles in *Enterobacteriaceae* isolated from the hide and skin samples.

Sample	Isolates	Multi-drug resistance	Isolate numbers
HS1[a]	*E.cloacae*	**8 R**[c] (AMC, CTR, CAZ, CXM, SXT, AZT, AMP, NAL); **1 I**[d] (CHL[e])	8
	E.coli	**0 R**; **1 I** (CEP)	
	E.vulneris	**1 R** (IMI); **5 I** (TOB, KAN, CAZ, CEP, OFX)	
	E.americana	**3 R** (CTR, CAZ, AZT); **5 I** (CXM, AMP, CHL, NAL, TET)	
	K. pneumoniaea ssp. ozaenae	**12 R** (AMK, KAN, AMC, AMS, PZT, FOX, CTR, CEP, CXM, SXT, AMP, NAL); **1 I** (AZT)	
	S.odorifera	**12 R** (KAN, GEN, AMC, AMS, FOX, CTR, CEP, CXM, SXT, AMP, CHL, TET); **1 I** (NAL)	
	S.rubidaea	**7 R** (AMS, PZT, FOX, AZT, AMP, CHL, NAL); **2 I** (KAN, CAZ)	
	Y. enterocolitica	**9 R** (TOB, CTR, CAZ, CXM, SXT, AZT, AMP, CHL, NAL); **3 I** (KAN, AMS, CEP)	
HS2	*C.lapagei*	**7 R**(PZT, CTR, CAZ, CXM, CIP, AZT, CHL); **1 I**(NAL)	5
	E.cloacae	**8 R** (AMC, CTR, CAZ, CXM, SXT, AZT, AMP, NAL); **1 I** (CHL)	
	P.vulgaris	**2 R** (PZT, AZT); **2 I** (STR, KAN)	
	S.rubidaea	**7 R** (AMS, PZT, FOX, AZT, AMP, CHL, NAL); **2 I** (KAN, CAZ)	
	Y.enterocolitica	**9 R** (TOB, CTR, CAZ, CXM, SXT, AZT, AMP, CHL, NAL); **3 I** (KAN, AMS, CEP)	
HS3	*C. lapagei*	**7 R** (PZT, CTR, CAZ, CXM, CIP, AZT, CHL) **1 I** (NAL)	5
	E. sakazakii	**11 R** (STR, AMC, AMS, PZT, CTR, CAZ, CEP, CXM, AZT, AMP, CHL); **2 I** (IMI, NAL)	
	S.liquefaciens	**4 R** (AMS, PZT, AZT, AMP); **0 I**	
	S.plymuthica	**5 R** (AMC, CTR, CXM, AZT, TET); **5 I** (PZT, CAZ, AMP, CHL, NAL)	
	S.rubidaea	**7 R** (AMS, PZT, FOX, AZT, AMP, CHL, NAL); **2 I** (KAN, CAZ)	
HS4	*C.lapagei*	**7 R**(PZT, CTR, CAZ, CXM, CIP, AZT, CHL); **1 I** (NAL)	5
	E.americana	**3 R** (CTR, CAZ, AZT); **5 I** (CXM, AMP, CHL, NAL, TET)	
	K.oxytoca	**0 R**; 2I (STR, CEP)	
	P.vulgaris	**2 R** (PZT, AZT); **2 I** (STR, KAN)	
	Y.enterocolitica	**9 R** (TOB, CTR, CAZ, CXM, SXT, AZT, AMP, CHL, NAL); **3 I** (KAN, AMS, CEP)	
HS5	*E.coli*	**0 R**; **1 I** (CEP)	4
	K.oxytoca	**0 R**; **2 I** (STR, CEP)	
	R.planticola	**2 R** (AZT, NAL); **3 I** (STR, CAZ, CXM)	
	R.ornithinolytica	**6 R** (CTR, CAZ, CXM, AZT, AMP, NAL); **2 I** (CEP, CHL)	
SS1[b]	*R.planticola*	**2 R** (AZT, NAL); **3 I** (STR, CAZ, CXM)	4
	S.odorifera	**12 R** (KAN, GEN, AMC, AMS, FOX, CTR, CEP, CXM, SXT, AMP, CHL, TET); **1 I** (NAL)	
	S.liquefaciens	**4 R** (AMS, PZT, AZT, AMP); **0 I**	
	S.ficaria	**10 R** (AMK, STR, TOB, KAN, GEN, AMC, MRP, AZT, AMP, TET); **3 I** (CAZ, CHL, NAL)	

Table I continued on following page.

Table I continued.

SS2	*E.cloacae*	**8 R** (AMC, CTR, CAZ, CXM, SXT, AZT, AMP, NAL); **1 I** (CHL)	5
	E.coli	**0 R**; **1 I** (CEP)	
	E.vulneris	**1 R**(IMI); **5 I** (TOB, KAN, CAZ, CEP, OFX)	
	S.plymuthica	**5 R**(AMC, CTR, CXM, AZT, TET); **5 I** (PZT, CAZ, AMP, CHL, NAL)	
	S.rubidaea	**7 R**(AMS, PZT, FOX, AZT, AMP, CHL, NAL); **2 I** (KAN, CAZ)	
SS3	*E.cloacae*	**8 R** (AMC, CTR, CAZ, CXM, SXT, AZT, AMP, NAL); **1 I** (CHL)	8
	E.coli	**0 R**; **1 I** (CEP)	
	K.oxytoca	**0 R**; **2 I** (STR, CEP)	
	P.vulgaris	**2 R** (PZT, AZT); **2 I** (STR, KAN)	
	P.penneri	**10 R** (STR, PZT, CTR, CAZ, CEP, CXM, OFX, SXT, AZT, CHL); **3 I** (KAN, AMP, NAL)	
	S.marcescens	**7 R** (STR, AMS, CEP, CXM, AZT, NAL, TET); **0 I**	
	S.rubidaea	**7 R** (AMS, PZT, FOX, AZT, AMP, CHL, NAL); **2I** (KAN, CAZ)	
	Y.enterocolitica	**9 R** (TOB, CTR, CAZ, CXM, SXT, AZT, AMP, CHL, NAL); **3 I** (KAN, AMS, CEP)	
SS4	*E.coli*	**0 R**; **1 I** (CEP)	4
	S.plymuthica	**5 R** (AMC, CTR, CXM, AZT, TET); **5 I** (PZT, CAZ, AMP, CHL, NAL)	
	S.marcescens	**7 R** (STR, AMS, CEP, CXM, AZT, NAL, TET); **0 I**	
	S.rubidaea	**7 R** (AMS, PZT, FOX, AZT, AMP, CHL, NAL); **2 I** (KAN, CAZ)	
SS5	*C.koseri*	**4 R** (TOB, PZT, CAZ, CEP); **5 I** (CXM, SXT, AZT, AMP, NAL)	7
	E.coli	**0 R**; **1 I** (CEP)	
	K.pneumoniaea ssp. ozaenae	**12 R** (AMK, KAN, AMC, AMS, PZT, FOX, CTR, CEP, CXM, SXT, AMP, NAL), **1 I** (AZT)	
	P.vulgaris	**2 R** (PZT, AZT); **2 I** (STR, KAN)	
	S.plymuthica	**5 R** (AMC, CTR, CXM, AZT, TET); **5 I** (PZT, CAZ, AMP, CHL, NAL)	
	S.rubidaea	**7 R** (AMS, PZT, FOX, AZT, AMP, CHL, NAL); **2 I** (KAN, CAZ)	
	Y.enterocolitica	**9 R** (TOB, CTR, CAZ, CXM, SXT, AZT, AMP, CHL, NAL); **3 I** (KAN, AMS, CEP)	

[a]HS1-HS5: Hide Samples 1-5,
[b]SS1-SS5: Skin Samples 1-5,
[c]R: Resistant,
[d]I: Intermediate
[e]CHL: chloramphenicol (30μg), AMK: amikacin (30μg), STR: streptomycin (10μg), TOB: tobramycin (10μg), KAN: kanamycin (30μg), GEN: gentamicin (10μg), AMC: amoxycillin-clavulanate (20/10μg), AMS: ampicillin-sulbactam (10/10μg), PZT: piperacillin-tazobactam (110μg), IMI: imipenem (10μg), MRP: meropenem (10μg), FOX: cefoxitin (30mg), CTR: ceftriaxone (30mg), CAZ: ceftazidime (30mg), CEP: cephalothin (30mg), CXM: cefuroxime sodium (30mg), CIP: ciprofloxacin (5μg), NOR: norfloxacin (10μg), OFX: ofloxacin (5μg), SXT: trimethoprim-sulfamethoxazole (1.25/23.75μg), AZT: aztreonam (30μg), AMP: ampicillin (10mg), NAL: nalidixic acid (30μg), TET: tetracycline (30μg).

R. ornithinolytica strains, isolated from termites, fish and ticks, were found to be resistant to nalidixic acid but susceptible to ciprofloxacin.

Strains of *Enterobacter cloacae* (HS1, HS2, SS2, SS3), *Proteus vulgaris* (HS2, HS4, SS3, SS5), *Raoultella planticola* (HS5, SS1), *Serratia liquefaciens* (HS3, SS1), *Serratia plymuthica* (HS3, SS2, SS4, SS5), *Serratia rubidaea* (HS1, HS2, HS3, SS2, SS3, SS4, SS5) and *Yersinia enterocolitica* (HS1, HS2, HS4, SS3, SS5), which were isolated from both hide and skin samples, were found to be resistant against eight, two, two, four, five, seven and nine antimicrobial agents, respectively (Tables I and II).

Strains of *Enterobacter cloacae* exhibited resistance to ampicillin, amoxycillin-clavulanate, ceftriaxone, ceftazidime, cefuroxime sodium, trimethoprim/sulfamethoxazole, aztreonam and nalidixic acid (Tables I and II). In another study, the intrinsic resistance of *E. cloacae* to cefoxitin, ampicillin, amoxycillin-clavulanate and ampicillin-sulbactam was stated by researchers.[33]

Strains of *Proteus vulgaris* and *Raoultella planticola* were fairly susceptible to most of the antibiotics tested. While *Proteus vulgaris* was resistant to piperacillin-tazobactam and aztreonam, this study found *R. planticola* was resistant to aztreonam and nalidixic acid. In another experiment, researchers reported intrinsic resistance of *P. vulgaris* against cefuroxime, ampicillin and tetracycline.[33] Although *P. vulgaris* was found resistant to piperacillin-tazobactam in our study, this species was susceptible to piperacillin-tazobactam in research conducted by Alhambra et al. (2004). In the previous study, *R. planticola*, which causes soft-tissue infection, was found resistant to amoxicillin but susceptible to amoxicillin-clavulanate, ciprofloxacin, cephalosporins and aminoglycosides.[29]

Strains of *Serratia liquefaciens* were found resistant to ampicillin-sulbactam, piperacillin-tazobactam, aztreonam and ampicillin. In the study of Sala et al. (2012), *S. liquefaciens* isolated from beef carcasses was found to be resistant to ampicillin.

Strains of *Serratia plymuthica* demonstrated resistance to amoxycillin-clavulanate, ceftriaxone, cefuroxime sodium, aztreonam and tetracycline (Tables I and II). In another investigation, *S. plymuthica* was found to be resistant to cefuroxime.[38]

Strains of *Serratia rubidaea* were resistant to ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, cefoxitin, aztreonam, chloramphenicol and nalidixic acid in our study. *S. rubidaea* isolated from clinical specimen was resistant to cefazolin and cefuroxime.[39]

Strains of Y*ersinia enterocolitica* were resistant to tobramycin, ceftriaxone, ceftazidime, cefuroxime sodium, trimethoprim-sulfamethoxazole, aztreonam, ampicillin, chloramphenicol and nalidixic acid (Tables I and II). *Y. enterocolitica* isolated from pig farms was found to be resistant to ampicillin and sulfamethoxazole.[40]

Among the test strains, *Klebsiella pneumoniaea* ssp. *ozaenae* (HS1, SS5) and *Serratia odorifera* (HS1, SS1) showed the highest multidrug resistance (12 antibiotics). Both isolates were resistant to kanamycin, ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefoxitin, ceftriaxone, cephalothin, cefuroxime sodium and trimethoprim-sulfamethoxazole. While strains of *K. pneumoniaea* ssp. *ozaenae* were resistant to amikacin, piperacillin-tazobactam and nalidixic acid, resistance to tetracycline, chloramphenicol and gentamicin was detected in strains of *S. odorifera* (Tables I and II). Stock and Wiedemann (2001) found *K. pneumoniaea* ssp. *ozaenae* strains, isolated from clinical specimens and environment, to be resistant to amoxicillin. In other research, *S. odorifera* isolated from clinical specimens was found to be resistant to piperacillin, ampicillin, carbenicillin, tetracycline, chloramphenicol and cephalothin.[42]

Although antibiotic-resistant *Escherichia coli* strains have become a major and rapidly increasing problem in humans[17], the lowest antimicrobial resistance was detected in *E.coli* (HS1, HS5, SS2, SS5) strains isolated from salted cattle hide and sheep skin samples in our study. *E. coli* strains were susceptible to 23 antimicrobial agents tested but this strain was intermediate susceptible to only cephalothin (Tables I and II).

Researchers have analyzed the antibiotic resistance of *E. coli* strains, isolated from 29 beef farms. From the fecal samples on 28 of the 29 farms, 31% of isolates from feedlots (n=993) and 12% of isolates from cow-calf farms (n=807) were found to be resistant to one or more of 16 antimicrobials used. Although, 1% of the isolates were resistant to ceftiofur, all *E.coli* isolates were susceptible to ceftriaxone, ciprofloxacin, gentamicin and nalidixic acid.[43]

In addition to *E.coli* strains, *Klebsiella oxytoca* (HS4, HS5, SS3) and *Escherichia vulneris* (HS1 and SS2) strains also showed low antimicrobial resistance. Our study showed *K. oxytoca* strains were susceptible to 22 antibiotics but intermediate susceptible to streptomycin and cephalothin (Tables I and II). However, Yigit et al. (2003) reported that K. oxytoca was resistant to imipenem, meropenem, extended-spectrum cephalosporins and aztreonam. Strains of *E. vulneris* were susceptible to 18 antibiotics but these strains were intermediate susceptible to tobramycin, kanamycin, ceftazidime, cephalothin and ofloxacin. Although a high percentage of the isolates (almost 95%) was susceptible to imipenem, interestingly strains of *E. vulneris* showed only resistance to imipenem (Tables I and II). *E. vulneris,* isolated from human wounds, was found to be resistant to penicillin and clindamycin in the study of Brenner et al. (1982).

Conclusion

This is the first study to examine prevalence of antibiotic-resistant members of the family *Enterobacteriaceae* isolated from salted cattle hides and sheep skins. Interestingly, multidrug-resistant members of the family *Enterobacteriaceae* was observed at all hide and skin samples. The source of these multidrug-resistant bacteria was thought as animals' intestine. While most of the isolates tested were susceptible to amikacin, imipenem, meropenem, ofloxacin, ciprofloxacin, tobramycin, gentamicin, trimethoprim-sulfamethoxazole, cefoxitin, amoxycillin-clavulanate, streptomycin, kanamycin, cephalothin and tetracycline, all isolates were susceptible to norfloxacin. Resistance to both highly important antimicrobials and critically important antimicrobials with highest priority[17] was observed among the our test isolates. The most important conclusion of this study was detection of resistance of our test isolates to these important antimicrobials. Hence, effective antimicrobial applications should be applied during preservation of skin and hides to eradicate these antibiotic-resistant bacteria in the leather industry.

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