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Bacterial Pathogens Recovered from Domestic Animals and their *in Vitro* Susceptibility Testing to Twelve Antimicrobial Agents

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ABSTRACT. A bacteriological examination of 112 necropsy tissue specimens (livers, spleens, lungs, lymph nodes) from domestic animals including cows, sheep, goats and chickens revealed that 55 (49.11%) of the samples were positive for bacteria. The 9 bacterial genera most commonly encountered and their incidences were as follows: *Escherichia* (15.18%), *Pseudomonas* (8.93%), *Pasteurella* (*Past. haemolytica* 8.04% and *Past. multocida* 0.89%), *Streptococcus* (4.46%), *Corynebacterium* (4.46%), *Proteus* (2.68%), *Salmonella* (2.68%), *Klebsiella* (0.89%) and *Staphylococcus* (*S. aureus* 0.89%).

Of 61 mastitic milk samples examined, 44 (72.13%) showed bacterial contamination. *Escherichia coli* was the predominant potential pathogen in milk followed by 6 other bacterial floras, namely, *Staphylococcus aureus*, *Streptococcus*, *Alcaligenes feacalis*, *Pseudomonas aeruginosa*, *Proteus* and *Klebsiella pneumoniae*.

Susceptibility testing of the bacterial isolates to 12 antimicrobial agents showed that the five most effective drugs tested were chloramphenicol, furazolidone, nitrofurantoin, sulfathiazole and polymyxin.

There is a growing concern about the importance of animal diseases to the national economies of all countries, particularly in developing nations. The United States Department of Agriculture estimated the cost of livestock losses in the United States (based on average sale) for cattle, swine and sheep diseases amounting to 1221 million dollars (USDA 1954). To our knowledge, there is no adequate system of reporting animal morbidity and mortality, nor any extensive survey on causes of animal diseases in Saudi Arabia. A few reports are published sporadically explaining the epidemiology of some of the widespread epidemics in an area such as the 'Rinder-like disease' observed in goats and sheep near Hofuf (Razig *et al.*

1981), the profile of major medical disease conditions observed in domestic animals in Al-Hassa (Razig and Parvez 1981) and infectious bovine rhinotracheitis and salmonellosis in Saudi Arabian dairy herds (Frerichs *et al.* 1982).

The bacteriological examination of necropsy material from tissues of the infected animals is of utmost importance in the confirmation of the potential etiology of suspected disease. Bacteriology is one of the fundamental fields in the areas of epidemiology and veterinary health. The results of bacterial examinations are used often in confirming diagnoses, establishing prognoses and selecting effective treatment based on reliable antimicrobial susceptibility testing.

This study documents the results of the bacteriological examination of necropsy materials and mastitic milk samples of putative diseased animals and presents the results of the susceptibility testing of the bacterial isolates.

Material and Methods

Bacteriological Examination

Necropsy tissue specimens consisting of livers, lungs, spleens and lymph nodes were obtained aseptically from necropsied animals, showing severe symptoms of a disease or freshly dead from a disease, and placed in sterile petri dishes. They were processed in the Animal Bacteriology Laboratory immediately after collection. In case of unavoidable delay, the specimens from freshly autopsied carcasses were refrigerated (4°C) and then cultured within 4 hr of their collection. Each organ was seared with a flamed spatula and a loopful of the internal tissue was streaked on a blood agar and a MacConkey agar media (Difco). The plates were incubated aerobically, at 37°C for 24 hr. Plates showing no detectable growth were incubated for an additional 24 hr.

The mastitic milk samples were submitted by dairy farm veterinarians. They were collected from the dairy cows after disinfecting the udder and teats with iodine solution. Twenty to 30 ml of the foremilk was discarded and then about 20 ml of the midstream milk was collected in a sterile universal bottle. The milk samples were transported in an ice chest to the Animal Bacteriology Laboratory. They were processed and cultured in the same manner as the necropsy samples.

The bacterial isolates from the examined samples were identified to the genus level and in certain cases, at the request of the veterinarian, to the species level, according to standard procedures (Lennette *et al.* 1974, Cottral 1978, Finegold *et al.* 1978).

Antimicrobial Susceptibility Testing

The purified bacterial cultures were maintained on stock culture medium slants (Difco) or stock culture slants supplemented with 5% sheep blood to support the

growth of fastidious organisms. The slants were then stored at 4°C to 6°C. Before testing, cultures were grown in nutrient or Avery's broth (nutrient broth supplemented with 5% sheep blood) for 4 hr at 37°C. A volume of 0.1 ml of the diluted culture, equivalent to 10^6 organisms/ml, was then plated on plain Muller-Hinton agar plates (Difco). The medium was also supplemented with 5% sheep blood to help support growth of fastidious bacterial genera such as *Corynebacterium*, *Pasteurella* and *Streptococcus*. Disk diffusion tests were performed according to the method of Bauer *et al.* (1966).

The following antimicrobial agents (BBL) were used for testing the susceptibility of the bacterial isolates by the disk-agar diffusion method: Chloramphenicol (CM, 30 μg), Furazolidone (FX, 100 μg), Nitrofurantoin (NF, 300 μg), Sulfathiazole (ST, 0.25 mg), Ampicillin (AM, 10 μg), Polymyxin B (PB, 300 units), Cephaloridine (CD, 30 μg), Cephalothin (CF, 30 μg), Kanamycin (K, 30 μg), Tetracycline (TE, 30 μg), Streptomycin (SM, 10 μg), Penicillin (PN, 10 units).

Results

The bacteriological examinations of 112 necropsy specimens obtained from cows, sheep, goats and chickens revealed heavy bacterial growth from 55 (49.11%) of the samples. The bacterial isolates from freshly autopsied carcasses belonged to the following 9 genera, listed according to their predominance: *Escherichia*, *Pseudomonas*, *Pasteurella*, *Streptococcus*, *Corynebacterium*, *Proteus*, *Salmonella*, *Klebsiella* and *Staphylococcus* (Table 1). Isolation of such organisms from the specified organs of freshly autopsied carcasses indicated that agents involved were pathogenic to the infected animal.

Table 2 shows the bacteria recovered from the mastitic milk samples collected from cows and goats. The overall infection rate is 72.13%. The composite results of the bacteriological examination reveals that *Escherichia coli* is the most common organism (26.22%) recovered from the milk samples, followed by *Staphylococcus aureus*, *Streptococcus* spp., *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, *Proteus* spp. and *Klebsiella pneumoniae*.

The results of the *in vitro* susceptibility testing of 39 strains of bacteria to 12 antimicrobial agents are shown in Table 3.

The choice of drugs for the treatment of disease caused by each bacterium, based on *in vitro* susceptibility testing, is shown in Table 4.

Discussion

The wide distribution of various bacteria recovered from the examined necropsy and mastitic milk samples are presented in Tables 1 and 2. All of these bacteria are of veterinary importance as frank or potential pathogens causing a

wide spectrum of animal diseases (Barnum *et al.* 1967, Sojka 1965, NAS 1971, Heddleston *et al.* 1967, Cobb and Walley 1962). The 5 most common bacterial pathogens in necropsy materials were: *Escherichia coli*, *Pseudomonas aeruginosa*, *Pasteurella haemolytica*, *Streptococcus* spp. and *Corynebacterium* spp. The two

Table 1. Occurrence of bacterial potential pathogens in 112 necropsy tissue samples obtained from livestock and poultry in Saudi Arabia.

Bacterial isolate	No. of positive samples				% of infected samples
	Spleen	Liver	Lung	Lymph node	
Escherichia coli	6	5	3	3	15.18
Pseudomonas aeruginosa	3	3	2	2	8.93
Pasteurella haemolytica	7	–	1	1	8.04
Streptococcus spp.	1	1	3	–	4.46
Corynebacterium spp.*	–	–	3	2	4.46
Proteus spp.	1	2	–	–	2.68
Salmonella spp.	–	2	–	1	2.68
Klebsiella spp.	–	–	1	–	0.89
Pasteurella multocida	1	–	–	–	0.89
Staphylococcus aureus	–	1	–	–	0.89
Total	19	14	13	9	49.11

* Three out of 5 cultures were identified as *Corynebacterium pyogenes*.

Table 2. Bacterial isolates encountered in mastitic milk of cows and goats in Saudi Arabia.

Bacterial isolate	No. of positive samples		% of infected samples
	Cows*	Goats**	
Escherichia coli	16	–	26.22
Staphylococcus aureus	9	2	16.39
Streptococcus spp.	9	–	14.75
Alcaligenes faecalis	4	–	6.56
Pseudomonas aeruginosa	2	–	3.28
Proteus spp.	1	–	1.64
Klebsiella pneumoniae	–	1	1.64
Total	41	3	72.13

* Samples examined = 53
** Samples examined = 8

Table 3. Susceptibility testing of 39 bacterial isolates to 12 antimicrobial agents; Disk diffusion method.

Bacterial Strain	Number tested	No. of strains sensitive to antimicrobial agents*											
		CM	FX	NF	ST	AM	PB	CD	CF	K	TE	SM	PN
Escherichia coli	16	13	14	14	10	9	11	9	8	9	10	11	1
Streptococcus	8	6	4	4	2	3	1	3	3	5	4	2	2
Alcaligenes faecalis	4	2	–	–	3	2	2	–	1	3	1	1	–
Pasteurella haemolytica	4	4	4	4	3	4	4	4	4	2	3	2	–
Pseudomonas aeruginosa	3	–	–	–	2	–	1	–	–	1	1	–	–
Salmonella	2	1	2	2	–	2	2	2	2	–	–	–	–
Klebsiella	1	1	1	–	1	–	–	1	1	–	–	–	–
Staphylococcus aureus	1	1	1	1	1	1	–	1	1	–	–	1	–
Total	39	28	26	25	22	21	21	20	20	20	19	17	3

* (CM) Chloramphemicol, (FX) Furazolidone, (NF) Nitrofurantoin, (ST) Sulfathiazole, (AM) Ampicillin, (PB) Polymyxin, (CD) Cephaloridine, (CF) Cephalothin, (K) Kanamycin, (TE) Tetracycline, (SM) Streptomycin, (PN) Penicillin.

Table 4. Choice of drugs for each bacterial isolate based on *in vitro* susceptibility testing.

Bacterial Strain	Drug* (choice number)
Escherichia coli	FX,NF (1); CM (2); SM,PB (3); ST,TE (4); AM,K,CD (5); CF(6); PN (7).
Streptococcus	CM (1); K (2); NF,TE,FX (3); AM,CF,CD (4); PN,SM,ST (5); PB (6).
Alcaligenes faecalis	ST,K (1); CM,AM,PB (2); SM,TE,CF (3).
Pasteurella haemolytica	FX,CM,AM,PB,NF,CF,CD (1); ST,TE (2); SM,K (3).
Pseudomonas aeruginosa	ST (1); PB,TE,K (2).
Salmonella	FX,AM,PB,NF,CF,CD (1); CM (2).
Klebsiella	FX,CM,ST,CF,CD (1).
Staphylococcus aureus	FX,SM,CM,ST,AM,NF,CF,CD (1)

* (CM) Chloramphenicol, (FX) Furazolidone, (NF) Nitrofurantoin, (ST) Sulfathiazole, (AM) Ampicillin, (PB) Polymyxin, (CD) Cephaloridine, (CF) Cephalothin, (K) Kanamycin, (TE) Tetracycline, (SM) Streptomycin, (PN) Penicillin.

predominant bacterial species recovered from the liver specimens from infected animals were *Escherichia coli* and *Pseudomonas aeruginosa* whereas, the two predominant species encountered in spleenic tissue were *Pasteurella heamolytica* and *Escherichia coli* (Table 1). The above results emphasize the importance of examining various necropsy specimens from infected animals in order to obtain a maximum recovery rate. Osbaldiston (1973) recommended the examination of various necropsy specimens from his experience on examining clinical samples. Many veterinarians, cooperating with his laboratory, relied on the identification of the bacteria involved and susceptibility testing of isolates for diagnosis and therapy of the disease.

The predominant bacteria recovered from the mastitic milk samples were: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* spp., *Alkaligenes faecalis* and *Pseudomonas aeruginosa* (Table 2). Various reports on the identification of bacteria in mastitic milk samples have shown varying distribution profiles of different bacterial flora (Thieme and Haasmann 1978, Verma and Mishra 1977, Hamir *et al.* 1978). The identification and susceptibility testing of the bacterial isolates from mastitic milks are of vital importance for epidemiological and control purposes and for effective treatment of mastitis. Similar research projects with particular emphasis on the control of mastitis have been conducted in many parts of the world (Thieme and Haasmann 1978, Walker and Williams 1978).

The susceptibility testing of the 39 bacterial isolates from necropsy specimens from infected animals and milk samples from mastitic udders indicated that chloramphenicol was the most effective drug against most bacterial infections

(Table 3). The drug of choice for the treatment of infections caused by the isolated infectious bacteria is given in Table 4. Tables 3 and 4 indicate the difference in susceptibility of different bacteria to antimicrobial agents, which is in agreement with the work of Verma and Mishra (1977).

This study, to our knowledge, documents for the first time in the Kingdom of Saudi Arabia, the occurrence of a wide spectrum of pathogenic bacteria in necropsy material from infected animals and mastitic milk samples. It also shows the great importance of antimicrobial susceptibility testing of the bacterial isolates in order to help the veterinarians in their choice of the appropriate antibacterial therapy for the treatment of animal disease and also to determine the trends in the developments of bacterial drug resistance (Nabbut *et al.* 1981). For the treatment of bacterial diseases, it is best to select the drug to which bacteria are most susceptible *in vitro*.

There is an urgent need for the establishment of new, well-equipped and well-staffed diagnostic microbiology laboratories, and the continued development of those already in existence, in the various provinces of the Kingdom of Saudi Arabia. Such laboratories are of great importance for assisting field veterinarians in the diagnosis, treatment and control of animal diseases.

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الجراثيم المرضية المعزولة من الحيوانات الأليفة وحساسيتها لاثنى عشر نوعاً من المضادات الحيـــوية

إيلي كميل بربور ، نسيم حنا نبوت و حبيب مقبول النخلي

المركز الإقليمي لأبحاث الزراعة والمياه ـ وزارة الزراعة والمياه ـ

الرياض ـ المملكة العربية السعودية

أظهـر الفحص الجرثومي لـ ١١٢ عينة حيوان مشرح (كبد ، طحـال ، رئـة ، غدد لمفـاوية) من الأبقار ، الأغنام ، الماعز والدجاج أن ٥٥ (٤٩,١١ ٪) من مجموع العينات كان إيجابياً .

تم عزل ٩ أجنـــاس من الجـراثيـم حسـب الـنسب التـــاليـــة : *Escherichia* (١٥,١٨ ٪) ، *Pseudomonas* (٨,٩٣ ٪) ، *Past. haemolytica) Pasteurella* (٨,٠٤ ٪) ، *Past. multocida* (٠,٨٩ ٪) ، *Streptococcus* (٤,٤٦ ٪) ، *Corynebacterium* (٤,٤٦ ٪) ، *Proteus* (٢,٦٨ ٪) ، *Salmonella* (٢,٦٨ ٪) ، *Klebsiella* (٠,٨٩ ٪) ، *Staphylococcus* (٠,٨٩ ٪) .

من أصل ٦١ عينة حليب مجموعة من حيوانات مصابة بالتهاب الضرع وجد في ٤٤ (٧٢,١٣ ٪) جراثيم . كانت *Escherichia coli* الأكثـر شيـوعاً في قدرة إحداث المرض يتبعها

أنواع جراثيم أخرى كالتالي :

Staphylococcus aureus , Streptococcus , Alcaligenes faecalis , Pseudomonas aeruginosa , Proteus , Klebsiella pneumoniae

إن اختبار حساسية عشرات الجراثيم المعزولة لـ ١٢ نوعاً من المضادات الحيوية أظهر أن هناك خمسة أنواع من الأدوية الأكثر فعالية وهي : **كلورامفينيكول ، فيرازوليدون ، نيتروفورانتون ، سلفاثيازول ، والبوليميكسين .**