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Detection of Aerobic Bacterial Species Causing Pneumonia in Sheep and Goats and their Susceptibility to Antibiotics in Sudan

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was conducted in Khartoum and Gezira States slaughterhouses to investigate the bacterial spp. associated with pneumonia in condemned sheep and goat lungs.
Study Design: A total of 2085 animals were examined grossly for respiratory lesions (sheep= 1601 and goats= 484).
Place and Duration of Study: Khartoum and Gezira States slaughterhouses during different seasons, from 2018-2020 and conducted in Central Veterinary Research Laboratory, Sudan.
Methodology: Bacterial spp. were isolated and identified using analytical profile index strips then more characterization done using Polymerase chain reaction for representative 20 selected isolates and finally antimicrobial susceptibility test was performed.

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Results: Out of 320 pneumonic lung samples, 116 samples showed pure colonies and 41 were mixed culture while 163 revealed no bacterial growth. The main bacteria isolated were *Staphylococcus aureus, Escherichia coli, Pasteurella multocida, Mannheimia haemolytica, Streptococcus* spp*. Corynebacterium spp. Pseudomonas spp. Staph* spp. and *Bacillus* spp. Twenty representative bacterial isolates examined by PCR technique 10 were *Staph aureus* and 10 were *E*. *coli* to confirm identification. Seven of each were found positive. The Gram-positive bacteria resulted in 41 resistant isolates, 21 intermediate and 141 sensitive to different antibiotics. Ten bacterial species were reported resistant for more than two Antibiotics from different classes mainly tetracycline, methicillin, penicillin and erythromycin. The Gram-negative bacteria resulted in 59 were resistant, 13 intermediate and 108 sensitive to antibiotics. Seventeen of them were multidrug resistance to tetracycline, gentamicin, Imipenem and ceftriaxone.
Conclusion: It was concluded that bacteria could be the main cause responsible for pneumonia in sheep and goats, some of these bacterial isolates were resistance to several Antibiotic which have an impact on public health.

Keywords: Pneumonia; isolated bacteria; sheep; goats; AST; PCR; Sudan.

1. INTRODUCTION

Pneumonia is probably the most single killing condition of animals, the magnitude of problem may be much greater than anticipated; economic losses from this condition are due to death of animal or financial expenses related to treatment. The disease in sheep and goat causes loss of weight and increased predisposition to pleurisy resulting in significant losses due to depression of lamb´s growth rate and neonatal and adult animal mortalities [1].

The etiological agents of pneumonia are complex, usually a combination of stress factor that weaken animal immune system and hence helps microorganisms to proliferate before pneumonic changes are produced. These agents are mainly bacteria like *Mannheimia*, *Pasteurell*a, *Mycoplasma*, *Klebsiella*, *Bordetella, Haemophillus*, *Staphylococcus, Streptococcus, Corynebacterium, Chlaymydia* species and *Escherichia coli*. In Nigeria, aerobic bacteria isolated from the lungs with pneumonia were E. coli, *Klebsiella pneumoniae*, *Mannheimia haemolytica*, *Streptococcus pyogenes*, *Staphylococcus aureus*, Bacillus subtilis, Proteus vulgaris and *Pasteurella multocida*. Escherichia coli was the most predominant isolate with a prevalence rate of 73.5% [2].

2. MATERIALS AND METHODS

2.1 Isolation and Identification of Isolates

The study was conducted in Khartoum and Gezira States during three seasons (hot, cold, rainy). Sheep and goats brought to slaughter-houses of local breed of both sexes, and aged between 7 month – 3 years. Other samples were also taken from pathology department, Central Veterinary Research Laboratory which were mainly goats of foreign breed (saanin). A total of 230(sheep) and 90(goat) lungs were collected from 1601 slaughtered sheep and 484 slaughtered goat in Khartoum and Gezira States, Sudan. Specimens were taken aseptically from each lung tissue that showed lesions using sterile surgical scalpel blades; kept as fresh tissues in sterile plastic bags in icebox. The purified isolates were identified according to the criteria out lined by [3,4] and [5]. The isolates were characterized using standard bacteriological techniques and different Analytical profile index strips [6].

2.2 Characterization of *Staphylococcus aureus* and *E. coli* using PCR

DNA extraction was done by adding two ml of normal saline to each lyophilized bacteria and mixed gentle, then cultured and incubated. Then extracted by boiling method.

PCR protocol for Staphylococcus aureus and *E. coli* were conducted according to [7] and [8].

The Primers for identification of *Staphylococcus aureus* were:

Primer Name	Sequence 5' – 3'	Product size (bp)
Nuc F	GCGATTGATGGTGATACGGTT	270
Nuc R	AGCCAAGCCTTGACGAACTAAAGC	

Primers for identification of *E. coli* were:

Primer Name	Sequence 5' – 3'	Product size (bp)
yaioF	TGATTTCCGTGGGTCTTGAATG	115
yaioR	ATGCCTGCCGTAGGGTGTTT	

According to (6) A volume of one µl of each forward and reverse primer, 2 µl of 10x PCR buffer, 2 µl of dNTPs, 0.25µl of iTag polymerase enzyme (Intron, Seoul, South Korea) and 10.75 µl nuclease free water were added to 3 µl of the extracted DNA to get the final volume of 20 µl for the reaction in PCR eppendorf tube. DNA amplification was carried out for 30 cycle's reaction as followed:

For *Staphylococcus aureus*: Initial denaturation step at 94 ºC for 3 min, DNA denaturation at 95 ºC for 30 second, annealing at 55 ºC for 30 second and final extension at 72 ºC for 5min.

For *Escherichia coli*: Initial denaturation step at 94 ºC for 3 min, DNA denaturation at 95 ºC for 30 second, annealing at 60 ºC for 30 second and final extension at 72 ºC for 5 min. Then Agarose gels of 1% in 0.5x TBE buffer were used for electrophoresis of the PCR products. Red Safe (Intron, Seoul, Korea) stain was added to the gels and a power of 100 volt was applied on the gels in 0.5x TBE running buffer for 40 min. Visualization of the results was achieved under UV light trans-illuminator.

2.3 Antibiotics Susceptibility Test

Antibiotics susceptibility test was performed in fresh culture organisms according to [9].

2.4 Statistical Analysis

Data were subject to analysis of variance by Statistical Packages for Social Science (SPSS) software program version 21, using one way ANOVA, Chi square, The mean and their errors, mean separation were done by Duncan Multiple Range Test [10] and [11], The significance different was calculated at ($P<0.05$).

3. RESULTS

3.1 Isolation and Identification of Isolates

The percentages of pneumonia in sheep and goats were found to be 24.42 %, 27.07% respectively in Gezira state. In Khartoum State it was 10.07% in sheep, 13.53% in goats. Three hundred and twenty samples from 117 sheep and 49 goats which were collected from Gezira State slaughterhouses, and 113 sheep and 41 goats form Khartoum slaughterhouses. Out of 320 pneumonic lung samples, 116 cultures showed pure colonies and 41 samples showed mixed culture while 163 revealed no bacterial growth (128 Gram-positive and 69 Gram-negative). Out of 166 samples collected from Gezira State 73 samples yielded 94 bacteria (28 were Gram-negative and 66 were Gram-positive) and no bacterial growth was noticed in 93 samples. In Khartoum State 154 samples were collected 84 samples yielded 103 isolates (41 were Gram negative and 62 were Gram positive) and no bacterial growth in 70 samples. The main bacteria isolated were Staph aureus, *E.coli, P multocida, M. haemolytica*, *Streptococcus spp. Corynebacterium spp*. *Pseudomonas spp*. *Staph spp. and Bacillus* spp. The results were shown in Figs. 1 and 2.

3.2 Characterization of *Staphylococcus aureus* and *E. coli* using PCR

20 bacteria examined by PCR technique 10 were Staph aureus and 10 were E.coli. Seven of each were found positive. The details was described in Fig. 3.

3.3 Antibiotics Susceptibility Test

Gram positive bacteria showed that 41 resistant isolates, 21 intermediate and 141 were sensitive to different antibiotics. 10 bacterial species were resistant to more than two antibiotics from different classes [multidrug resistant] the results were described in Table 1.

The 24 Gram negative bacteria from the 59 resistant ones showed that 13 were intermediate and 108 were sensitive to antibiotics and 17 were multidrug resistance. The results were described in Table 2.

Most Gram positive bacteria were resistant to tetracycline, penicillin, methicillin, colistin and erythromycin, and they are highly sensitive to gentamicin, enrofloxacin and bacitracin. Furthermore most Gram negative bacteria were resistant to tetracycline, gentamicin, Imipenem and Ampicillin, however they are sensitive to ciprofloxacin and Amoxicillin only.

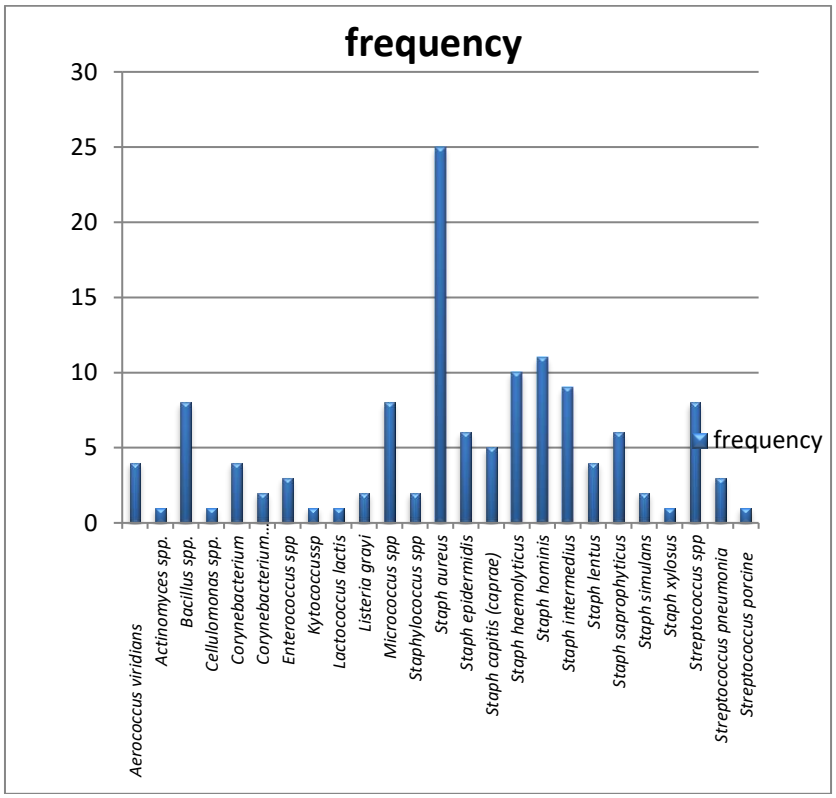

Fig. 1. Gram positive bacteria isolated from lung in Gezira and Khartoum States

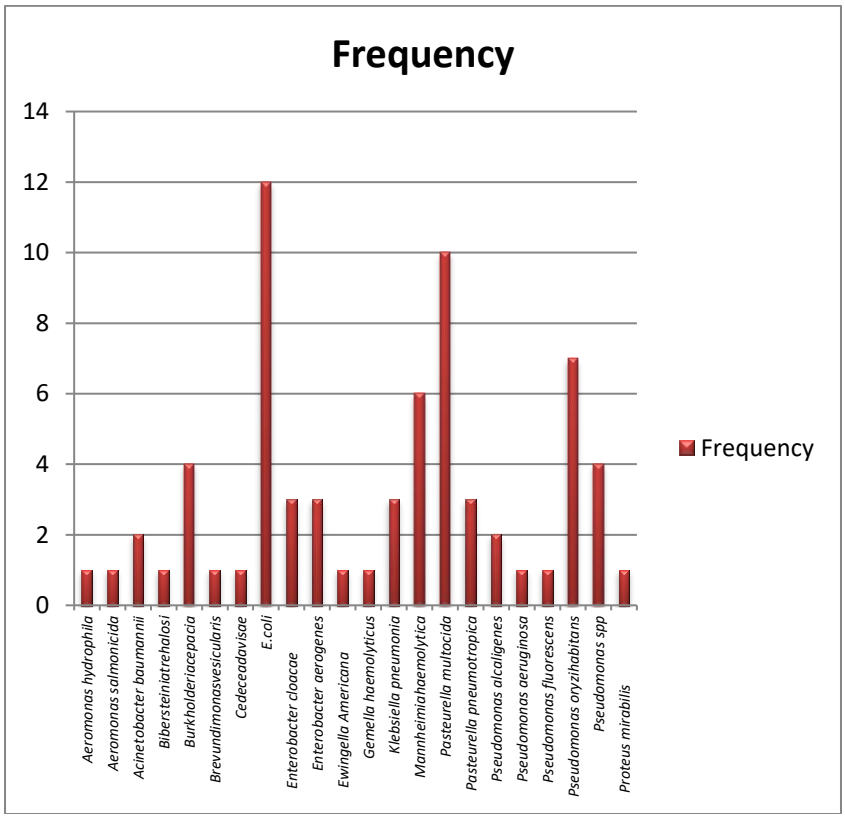

Fig. 2. Gram negative bacteria isolated from lung in Gezira and Khartoum States

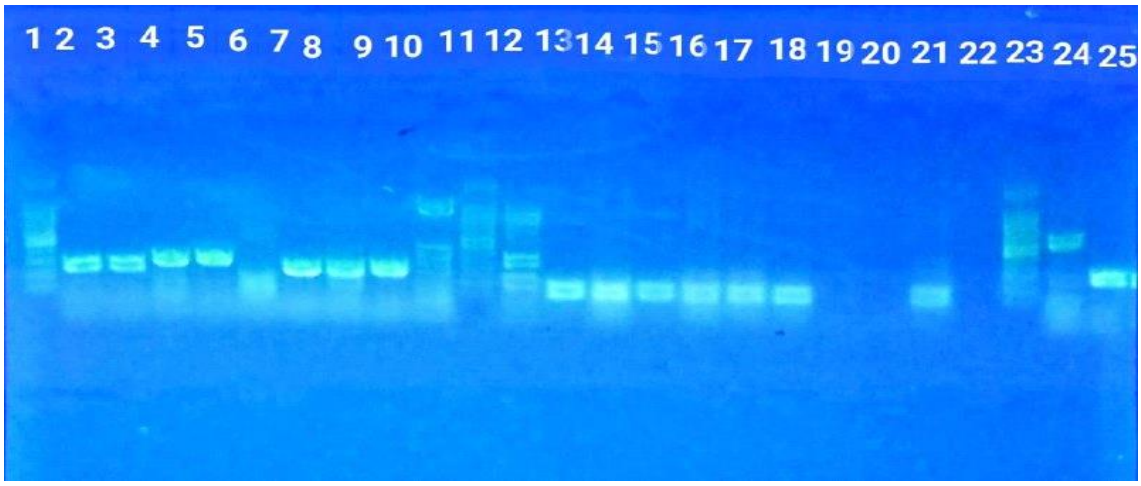

Fig. 3. Agrose gel electrophoresis of multiplex PCR product: 1, 12, 23=100 bp ladder, 2 positive control of Staph aureus, 3,4,5,7,8,9,25 are positive Staph aureus 13, 14, 15, 16, 17, 18, 21 positive *E. coli*

Table 1. Antimicrobial Susceptibility for Gram positive Bacteria

Bacteria	TE	ME	GEN	CT	NV	E	AX	P	CIP	VA	B	EX	CRO
s.aureus-1	R	R	S	R	S	S	I	R	-	S	-	-	-
s.aureus-2	S	S	S	-	-	R	I	S	S	S	-	-	-
s.aureus-3	S	S	S	-	R	S	S	I	S	S	-	-	-
s.aureus-4	S	S	S	S	S	I	-	S	S	S	S	-	-
s.simulans -1	S	S	S	-	-	S	-	R	S	-	S	S	-
s.simulans -2	S	S	S	-	-	S	-	S	I	S	-	I	-
s.hominus-1	S	S	-	S	-	R	-	S	-	S	S	S	-
S.hominus-2	S	-	-	R	-	I	-	S	I	-	I	S	-
s.intermid	S	S	S	-	S	R	-	S	-	S	-	I	-
S.lentus	S	-	S	-	-	R	-	R	S	-	-	S	S
S.lentus	S	S	S	-	-	I	-	S	S	-	I	S	S
s.hemolyti	S	S	S	-	S	S	-	S	-	S	-	S	-
S.epiderm	S	S	S	-	-	I	-	R	S	-	-	S	-
S.captis	S	S	S	-	-	S	-	R	S	-	-	S	-
S.lugdunenis	S	R	S	-	-	S	-	R	S			I	-
Micrococ-1	S	S	-	S	S	R	-	S	-	S	I	S	-
Micrococc-2	S	-	S	-	-	I	-	R	S	-	-	S	-
Micrococc-3	S	-	S	-	-	R	-	R	S	-	-	I	-
Micrococc-4	S	-	S	-	-	S	-	R	S	-	-	S	-
Enterococussp	S	-	S	-	-	I	-	S	S	-	-	S	-
Lacto.lactis	S	R	S	R	-	S	R	S	S	-	-	-	R
List grage-1	R	-	S	R	-	I	R	-	S	-	S	I	R
List grage-2	S	-	S	S	-	S	S	-	R	-	S	S	R
Strep.porcine	S	R	S	R	-	S	S	R	-	-	S	-	S
Erococus.veridans-1	R	-	S	R	-	I	-	S	R	-	-	-	R
Erococcus veridance-2	S	-	S	R	-	S	-	S	R	-	-	-	R

**S=Sensitive, I= Intermediate R= Resistance*

Table 2. Antimicrobial Susceptibility to Gram negative Bacteria

Bacteria /antibiotic tested	TE	GEN	C	CIP	CT	IPM	COT	CTX	CXM	AK	AMP	AX	CRO	EX	CAZ
E. coli-1	R	S	S	S	R	S	-	-	-	-	-	-	R	R	-
E. coli-2	S	S	R	S	R	R	-	-	-	-	-	-	R	I	-
E. coli-3	R	R	S	S	S	R	-	-	-	R	-	-	S	R	-
E. coli-4	S	I	-	S	-	R	-	R	-	-	-	-	R	S	-
E. coli-5	S	I	-	-	-	-	S	S	I	S	R	-	-	-	-
E. coli-6	S	R	-	-	-	-	S	S	R	S	R	-	-	-	-

Bacteria /antibiotic tested	TE	GEN	C	CIP	CT	IPM	COT	CTX	CXM	AK	AMP	AX	CRO	EX	CAZ
B. cepacia-1	R	R	-	-	-	R	R	S	S	S	S	S	S	-	-
B. cepacia-2	R	R	-	-	-	R	I	S	S	S	S	S	S	-	-
K. pneumoni1	I	S	-	I	-	R	-	-	-	-	-	S	R	-	-
K. pneumoni2	-	S	S	S	S	-	-	-	-	-	-	S	-	S	-
P. multocida	S	S	-	-	-	-	S	S	S	S	S	S	-	-	-
P. pneumoni	S	S	-	S	-	R	S	S	S	S	S	S	-	S	-
A.hydroph-1	S	S	-	S	-	R	-	-	-	-	-	-	S	I	-
A. hydroph-2	R	S	-	S	-	R	-	-	-	-	-	S	S	-	-
Ps.oryzihab.1	S		-	S	-	S	S	S	R	S	R	-	-	-	-
Ps.oryzihab.2	S	S	-	S	-	R	-	S	-	-	-	-	-	S	R
Ps. oryzihab.3	S	S	-	S	-	R	-	S	-	-	-	-	-	S	R
Ps. oryzihab.4	R	S	-	-	R	-	-	-	-	R	-	-	R	I	-
Shigella	S	S	S	S	-	R	-	R	-	-	-	-	-	S	S
Cedeceadavi	S	I	-	-	-	R	R	R	R	I	R	-	-	-	-
Enterobacter	S	S	-	-	S	R	-	-	-	S	-	-	R	R	-
E. amiricana	S	S	S	S	-	R	-	-	-	-	-	-	R	S	-
A. baumannii	S	S	-	I	-	R	-	R	-	-	-	-	-	I	R
Och.anthropi	S	S	S	S	-	-	-	-	-	-	-	-	R	S	-

**S=Sensitive, I= Intermediate R= Resistance*

3.4 Statistical Analysis

Correlations between bacterial isolated were found significant with state and season, and no significance relation with type of animal either sheep or goats (P<0.05).

Correlation/ Risk factor	P. value
Bacteria * State	.012
Bacteria * Season	.000
Bacteria * Type	.417

4. DISCUSSION

In this study 1601, 484 sheep and goat were examined respectively (2018-2020) with 76.8%, 23.2% percentage, as mention by animal health and epidemic control record 2018, the total number of sheep and goat slaughtered in Khartoum state were 755546, 281929 heads respectively. Sheep is highest percentage of slaughtered animal (54.4%) followed by cattle (24.7%) goat (20.3%) and camel 0.6% [12], this is the same in case of goat and differ in slaughtered sheep which is higher in my study.

A total of 196 bacterial isolates were isolated from the 320 pneumonic cultured lungs. 116 samples yield pure bacterial growth, 41 samples were mixed culture and no bacterial growth was noticed in 163 samples inspite of appearance with marked lesions grossly and histologically. Failure of bacterial isolation in these samples with observable pneumonic lesions may be due to different causes like mycobacterium, mycoplasma spp, anaerobicbacteria, and viral implication or may be due to antibiotic therapy that use before slaughter. This in accordance with a researcher from Iran who found some samples of pneumonic lungs with no bacterial growth [13].

The most bacteria isolated from Gezira State were Staph spp. *Pseudomonas spp. Pasteurella spp*. and *E.coli* while from Khartoum State were Staph spp. *Corynebacterium spp*. *Pasteurella* and *Mannheimia spp.*

Pasteurella spp and Mannheimia spp prevalence rates were 7.3% and 3.1% respectively. This finding was less than what reported by [14,15,16] who reported 11.67%, 11.8% and 15% respectively. Also less than a study done in El-Damazin area which report the percentage of *M. haemolytica and P. multocida* isolation from pneumonic sheep lung as 10.9% and 7.6% respectively [17].

Identification of the pneumonic pathogens in the present work cleared that Staphspp is predominant (42%), where Staph. aureus, was the most pneumonic bacteria isolated from lung tissue (12.9%). This result was in agreement with other investigator [16] and [14], who reported Staphspp 40% and 36.67% respectively. In contrast with [15] who reported that the percentage of Staphspp was (15.6%). in current study the percentage of Escherichia coli is 6.2%, this is lower than that reported in Nigeria which was found to be the most predominant isolate in goat with prevalence rate of 73.5% (2). other investigator [16] and [15] reported the

prevalences of E.coli 25%, 16.8% respectively in goat.

Mannheimia haemolytica and *Pasteurella spp*. were isolates herein with prevalence 10.4%, these bacteria were predominant (83.3%) in Khartoum State in 2012. In our study, six isolate of *Corynebacterium spp*. with percentage (3.1%).this is lower than [18] who found Coryn pseudotuberculosis 6.9% and haemolytic Streptococci 4.9%. Past multocida, Staph hyicus, Staph. caseolyticus, Staph. saccharolyticus and Actiomyces, Coryn pyogenes were represented 0.98% [18]. In my study different type of bacteria isolated, this is simillar in Ethiopia which 29 isolates of bacteria were detected from pneumonic lung of small Ruminants, namely *Pasteurella spp*. (47.85%) Staph spp. (17.68%) Strep spp. (13.44%) and other bacteria (21.03%) [19].

Herein our study for gram positive bacteria, out of 15 Staph spp. tested six were resistance to penicillin, four to erythromycin two to methicillin and one to tetracycline and they were sensitive for Gentamicin, vancomycin and enrofloxacin. Out of four micrococcus spp. tested three were resistance for penicillin two to erythromycin and sensitive to tetracycline, Gentamicin and ciprofloxacin. *Erococcus spp*. isolated was resistant to ciprofloxacin, tetracycline and colistin but sensitive to penicillin and Gentamicin. Out of six isolates of Gram negative bacteria three E.coli were resistance to Imipenem, ceftriaxone two to tetracycline, gentamicin, colistin, enrofloxacin and sensitive to ciprofloxacin. Two isolates of B. cepacia were resistance to gentamicin, tetracycline, Imipenem and sensitive to ceftriaxone, cefotaxime, cefuroxime. *Pasteurella spp* were sensitive for most of antibiotic tested except to Imepenm. *Pseudomonas spp* were resistance to CAZ and Imipenem and sensitive to gentamicin, ciprofloxacin and slightly sensitive to tetracycline. [16] found that Staphylococcus spp. was slightly sensitive to penicillin while *Pasteurellaspp* and E. coli were resistant to penicillin and *Staphylococcus spp., Pasteurella spp. and E. coli* were highly sensitive to oxytetracycline, streptomycin, kanamycin and ciprofloxacin. They were partially resistant to amoxicillin. *Staphylococcus spp* was sensitive to nalidixic acid while *Pasteurella spp*. and E. coli were completely resistant to nalidixic acid [16]. Past multocida were found highly sensitive to ciprofloxacin and resistant to penicillin. In Bangladesh Staph. aureus reported highly sensitive to erythromycin, tetracycline, enrofloxacin, and norfloxacin and less sensitive to amoxicillin [20].

5. CONCLUSION

This investigation has shown that pneumonia is one of the major causes for lung condemnation in slaughterhouses in both Khartoum and Gezira States.

Bacterial agents are the main cause of pneumonia with high incidence records mainly Staphaureus, *Pasteurella spp*, Manhaemolytica and *E. coli*.

It was concluded that bacteria could be the main cause responsible for pneumonia in sheep and goats, some of these bacteria is resistance to several antibiotic which have an impact on public health.

From 22 different Antibiotics, 383 single antibiotic discs were used against 50 different bacteria, 27 bacteria describe as multidrug resistance.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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