

Detection of aerobic bacterial species causing pneumonia in sheep and goats and their susceptibility to antibiotics in Sudan

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, from 2019-2020 and conducted in Central Veterinary Research Laboratory, Sudan.

Methodology: Bacterial spp were isolated and identified using analytical profile index strips (API) then more characterization done using Polymerase chain reaction (PCR) for representative 20 selected isolates and finally antimicrobial susceptibility test was performed.

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*, *E.coli*, *Pasteurella multocida*, *Mannheimia haemolytica*, *Streptococcus spp*, *Corynebacterium spp*, *Pseudomonas spp*, *Staph spp* and *Bacillus spp*. 20 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 two or more 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 (MDR) to tetracycline, gentamicin, Imepenm 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.

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Antibiotics not antibiotic :[4H]Comment because with several plural noun should be written

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 aetiological 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*, *Pasteurella*, *Mycoplasma*, *Klebsiella*, *Bordetella*, *Haemophilus*, *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Chlamydia* 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*. *E. 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 and 90 lungs were collected from 1601,484 slaughtered sheep and goat respectively 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 outlined by [3] [4] and [5]. The isolates were characterized using standard bacteriological techniques and different API 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 gently, then cultured and incubated. Then extracted by boiling method.

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

The Primers for identification of *Staphylococcus aureus* were:

Nuc F- GCGATTGATGGTGATACGGTT

Nuc R- AGCCAAGCCTTGACGAACTAAAGC

Primers for identification of *E. coli* were:

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start with capital letter, so write Specimens

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product of this primer?
You should mention for example it is 200bp

Yaio F- TGATTTCCGTGGGTCTTGAATG

Yaio R- ATGCCTGCCGTAGGGTGTTT

According to (6) A volume of one μ l of each forward and reverse primer, 2 μ l of 10x PCR buffer, 2 μ l of dNTPs, 0.25 μ l of iTag polymerase enzyme (Intron, Seoul, South Korea) and 10.75 μ l nuclease free water were added to 3 μ l of the extracted DNA to get the final volume of 20 μ l 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 °C for 3 min, DNA denaturation at 95 °C for 30 second, annealing at 55 °C for 30 second and final extension at 72 °C for 5min.

For *Escherichia coli* :

Initial denaturation step at 94 °C for 3 min, DNA denaturation at 95 °C for 30 second, annealing at 60 °C for 30 second and final extension at 72 °C 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 [8].

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 [9] and [10], 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. Out of 166 samples collected

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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 Table 1.

Table 1: Bacteria isolated from lung in Gezira and Khartoum State:

Gram positive bacteria	No.	Gram negative bacteria	No.
<i>Aerococcus viridians</i>	4	<i>Aeromonashydrophila</i>	1
<i>Actinomycessp</i>	1	<i>Aeromonassalmonicida</i>	1
<i>Bacillus</i> spp	8	<i>Acinetobacterbaumannii</i>	2
<i>Cellulomonassp</i>	1	<i>Bibersteiniatrehalosi</i>	1
<i>Corynebacterium</i>	4	<i>Burkholderiacepacia</i>	4
<i>Corynebacterium</i> pseudotuberculosis	2	<i>Brevundimonasvesicularis</i>	1
<i>Enterococcus</i> spp	3	<i>Cedeceadavisae</i>	1
<i>Kytococussp</i>	1	<i>E.coli</i>	12
<i>Lactococcuslactis</i>	1	<i>Enterobacter cloacae</i>	2
<i>Listeria grayi</i>	2	<i>Enterobacteraerogenes</i>	3
<i>Micrococcus</i> spp	8	<i>Ewingella Americana</i>	1
<i>Staphylococcus</i> spp	2	<i>Gemellahaemolyticus</i>	1
<i>Staph aureus</i>	25	<i>Klebsiella pneumonia</i>	3
<i>Staph epidermidis</i>	6	<i>Mannheimiahaemolytica</i>	6
<i>Staph capitis (caprae)</i>	5	<i>Pasteurellamultocida</i>	10
<i>Staph haemolyticus</i>	10	<i>Pasteurellapneumotropica</i>	3
<i>Staph hominis</i>	11	<i>Pseudomonas alcaligenes</i>	2
<i>Staph intermedius</i>	9	<i>Pseudomonas aeruginosa</i>	1
<i>Staph lentus</i>	4	<i>Pseudomonas fluorescens</i>	1
<i>Staph saprophyticus</i>	6	<i>Pseudomonas oryzihabitans</i>	7
<i>Staph simulans</i>	2	<i>Pseudomonas</i> spp	1
<i>Staph xylosus</i>	1	<i>Proteus mirabilis</i>	1
<i>Streptococcus</i> spp	8	-	
<i>Streptococcus pneumonia</i>	3	-	
<i>Streptococcus porcine</i>	1	-	
Total	128		65

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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 figure 1

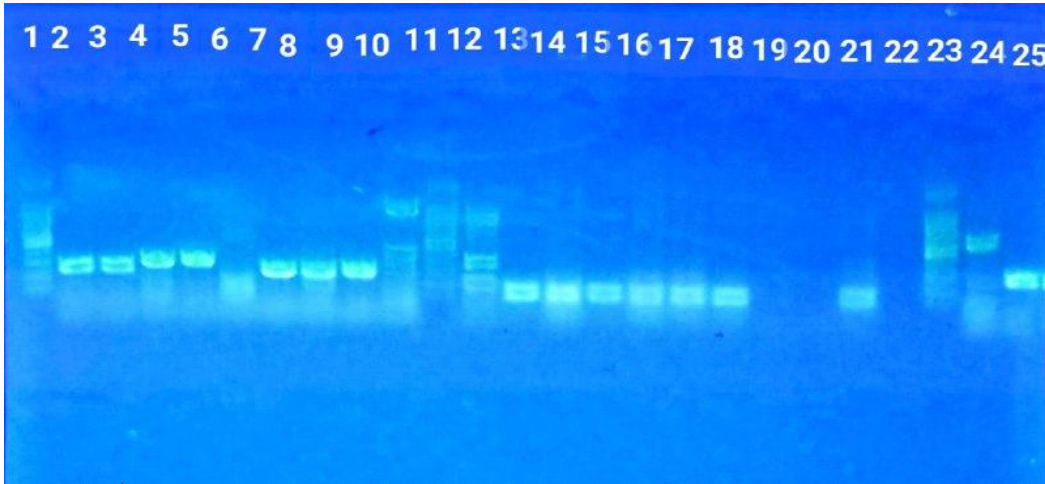


Fig (1): Agarose gel electrophoresis of multiplex PCR product: 1, 12, 23=100 bp ladder, 2 positive control of *Staph aureus*, 3,4,5,7,8,9,25 are positive *Staph aureus* 13, 14, 15, 16, 17, 18, 21 positive *E.coli*.

You should mention by arrow : [13H] Comment in the figure; the PCR products of *Staph* how many base pairs were!!! And the *E.coli* PCR products were how many base pairs

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 two or more antibiotics from different classes [multidrug resistant] the results were described in Table (2).

[14H] Comment Kindly note multidrug resistance is antimicrobial resistance shown by a species of microorganism to at least one antimicrobial drug in three or more antimicrobial categories. Focus on three or more but you wrote two or more!!!

Table 2: Antimicrobial Susceptibility for Gram positive Bacteria

Bacteria	TE	ME	GEN	CT	NV	E	AX	P	CIP	VA	B	EX	CRO
<i>s.aureus-1</i>	R	R	S	R	S	S	I	R	-	S	-	-	-
<i>s.aureus-2</i>	S	S	S	-	-	R	I	S	S	S	-	-	-
<i>s.aureus-3</i>	S	S	S	-	R	S	S	I	S	S	-	-	-
<i>s.aureus-4</i>	S	S	S	S	S	I	-	S	S	S	S	-	-
<i>s.simulans -1</i>	S	S	S	-	-	S	-	R	S	-	S	S	-
<i>s.simulans -2</i>	S	S	S	-	-	S	-	S	I	S	-	I	-
<i>s.hominus-1</i>	S	S	-	S	-	R	-	S	-	S	S	S	-
<i>S.hominus-2</i>	S	-	-	R	-	I	-	S	I	-	I	S	-
<i>s.intermid</i>	S	S	S	-	S	R	-	S	-	S	-	I	-
<i>S.lentus</i>	S	-	S	-	-	R	-	R	S	-	-	S	S
<i>S.lentus</i>	S	S	S	-	-	I	-	S	S	-	I	S	S
<i>s.hemolyti</i>	S	S	S	-	S	S	-	S	-	S	-	S	-
<i>S.epiderm</i>	S	S	S	-	-	I	-	R	S	-	-	S	-
<i>S.captis</i>	S	S	S	-	-	S	-	R	S	-	-	S	-

<i>S.lugdunensis</i>	S	R	S	-	-	S	-	R	S			I	-
<i>Micrococc-1</i>	S	S	-	S	S	R	-	S	-	S	I	S	-
<i>Micrococc-2</i>	S	-	S	-	-	I	-	R	S	-	-	S	-
<i>Micrococc-3</i>	S	-	S	-	-	R	-	R	S	-	-	I	-
<i>Micrococc-4</i>	S	-	S	-	-	S	-	R	S	-	-	S	-
<i>Enterococcus</i>	S	-	S	-	-	I	-	S	S	-	-	S	-
<i>Lacto.lactis</i>	S	R	S	R	-	S	R	S	S	-	-	-	R
<i>List grage-1</i>	R	-	S	R	-	I	R	-	S	-	S	I	R
<i>List grage-2</i>	S	-	S	S	-	S	S	-	R	-	S	S	R
<i>Strep.porcine</i>	S	R	S	R	-	S	S	R	-	-	S	-	S
<i>Erococcus.veridans-1</i>	R	-	S	R	-	I	-	S	R	-	-	-	R
<i>Erococcus veridance-2</i>	S	-	S	R	-	S	-	S	R	-	-	-	R

*S=Sensitive, I= Intermediate R= Resistance

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 (3).

Table 3: Antimicrobial Susceptibility to Gram negative Bacteria:

Bacteria /antibiotic tested	T E	GE N	C	CI P	C T	IP M	CO T	CT X	CX M	A K	AM P	A X	CR O	E X	CA Z
<i>E.coli-1</i>	R	S	S	S	R	S	-	-	-	-	-	-	R	R	-
<i>E.coli-2</i>	S	S	R	S	R	R	-	-	-	-	-	-	R	I	-
<i>E.coli-3</i>	R	R	S	S	S	R	-	-	-	R	-	-	S	R	-
<i>E.coli-4</i>	S	I	-	S	-	R	-	R	-	-	-	-	R	S	-
<i>E.coli-5</i>	S	I	-	-	-	-	S	S	I	S	R	-	-	-	-
<i>E.coli-6</i>	S	R	-	-	-	-	S	S	R	S	R	-	-	-	-
<i>B.cepacia-1</i>	R	R	-	-	-	R	R	S	S	S	S	S	S	-	-
<i>B.cepacia-2</i>	R	R	-	-	-	R	I	S	S	S	S	S	S	-	-
<i>K.pneumoni 1</i>	I	S	-	I	-	R	-	-	-	-	-	S	R	-	-
<i>K.pneumoni 2</i>	-	S	S	S	S	-	-	-	-	-	-	S	-	S	-
<i>P.multocida</i>	S	S	-	-	-	-	S	S	S	S	S	S	-	-	-
<i>P.pneumoni</i>	S	S	-	S	-	R	S	S	S	S	S	S	-	S	-
<i>A.hydroph-1</i>	S	S	-	S	-	R	-	-	-	-	-	-	S	I	-
<i>A.hydroph-2</i>	R	S	-	S	-	R	-	-	-	-	-	S	S	-	-
<i>Ps.oryzihab. 1</i>	S		-	S	-	S	S	S	R	S	R	-	-	-	-
<i>Ps.oryzihab. 2</i>	S	S	-	S	-	R	-	S	-	-	-	-	-	S	R
<i>Ps.oryzihab. 3</i>	S	S	-	S	-	R	-	S	-	-	-	-	-	S	R
<i>Ps.oryzihab. 4</i>	R	S	-	-	R	-	-	-	-	R	-	-	R	I	-
<i>Shigella</i>	S	S	S	S	-	R	-	R	-	-	-	-	-	S	S

<i>Cedeceadavi</i>	S	I	-	-	-	R	R	R	R	I	R	-	-	-	-
<i>Enterobacter</i>	S	S	-	-	S	R	-	-	-	S	-	-	R	R	-
<i>E. amiricana</i>	S	S	S	S	-	R	-	-	-	-	-	-	R	S	-
<i>A.baumannii</i>	S	S	-	I	-	R	-	R	-	-	-	-	-	I	R
<i>Och.anthropi</i>	S	S	S	S	-	-	-	-	-	-	-	-	R	S	-

*S=Sensitive, I= Intermediate R= Resistance

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, Imepenm and Ampicillin, however they are sensitive to ciprofloxacin and Amoxicillin only.

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methicillin

It should be written Imipenem : [16H]Comment

It should be : [17H]Comment
written Amoxicillin

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% [11], this is the same in case of goat and differ in slaughtered sheep which is higher in my study.

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So which one is the most accurate?
Kindly write the correct one

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*, anaerobic bacteria, 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 [12].

histologically : [19H]Comment
Correct the word, please

The most bacteria isolated from Gezira State were *Staphspp*, *Pseudomonasspp*, *Pasteurellasp* and *E.coli* while from Khartoum State were *Staph spp*, *Corynebacterium spp*, *Pasteurella* and *Mannheimiaspp*.

Pasteurellasp and *Mannheimiaspp* prevalence rates were 7.3% and 3.1% respectively. This finding was less than what reported by [13]; [14], [15] who reported 11.67%, 11.8% and 15% respectively. also less than a study done in El-Damazin area which report the percentage of

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M. haemolytica and *P. multocida* isolation from pneumonic sheep lung as 10.9% and 7.6% respectively [16].

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 [15] and [13], who reported *Staphspp* 40% and 36.67% respectively. In contrast with [14] 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 [15] and [14] 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 [17] who found *Corynseudotuberculosis* 6.9% and haemolytic Streptococci 4.9%. *Pastmultocida*, *Staphhyicus*, *S. caseolyticus*, *S. saccharolyticus* and *Actiomyces* *Corynpyogenes* were represented 0.98% [17]. in my study different type of bacteria isolated ,this is similar in Ethiopia which 29 isolates of bacteria were detected from pneumonic lung of small Ruminants, namely *Pasteurella* spp (47.85%) *Staphsp* (17.68%) *Strepsp* (13.44%) and other bacteria (21.03%) [18].

Herein our study for gram positive bacteria, out of 15 *Staphspp* tested six were resistance to penicillin, four to erythromycin two to methecillin 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 Imepenm, ceftriaxone two to tetracycline, gentamicin, colistin, enrofloxacin and sensitive to ciprofloxacin. Two isolates of *B. cepacia* were resistance to gentamicin, tetracycline, Imepenm 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 imepenm and sensitive to gentamicin, ciprofloxacin and slightly sensitive to tetracycline. [15] found that *Staphylococcus* spp. was slightly sensitive to penicillin while *Pasteurella* spp 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 [15]. *Pastmultocida* 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 [19].

5. CONCLUSION:

percentage : [22H] Comment

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methicillin : [24H] Comment
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Imipenem : [25H] Comment
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Imipenem : [26H] Comment

Imipenem : [27H] Comment

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*, *pasteurellaspp*, *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 383 single antibiotic used against 50 different bacteria, 27 bacteria describe as multidrug resistance.

: [28H] Comment

What is this?

Are 383 antibiotics used!!!

This result (number) not mentioned in the results or anywhere in the research and directly mentioned in the conclusion!!!

Kindly rewrite this information with accurate way.

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