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MOLECULAR CHARACTERIZATION AND ANTIBIOTIC RESISTANCE PATTERNS AGAINST *E. COLI, P. AERUGINOSA, ENTEROBACTER SPP. AND KLEBSIELLA SPP.* IN HUMANS AND ANIMALS. FIRST EVER REPORTED STRAINS IN DIVISION SAHIWAL

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Abstract
Background: Antimicrobial medications have been crucial in both animals and humans to reduce infections and death rate. Antibiotic resistance is found in all bacteria including *E. coli, P. aeruginosa, Enterobacter spp. and Klebsiella spp.* To find out the effective medication is necessary to cure the disease resulted from bacteria. **Objectives:** The purpose of the study was to isolate, test antibiotic resistance patterns against bacteria and to find novel strains from division. **Methodology:** This study was conducted in accordance to the declarations of Helsinki. About 120 stool samples from humans and 100 fecal samples were collected from chicken and goat. Bacteria were isolated in laboratory. Biochemical tests were employed to identify bacteria. To test antibiotic susceptibility five antibiotics on humans and thirteen antibiotics on chicken and goat were used against bacteria. DNA was extracted and checks quantity by agarose gel electrophoresis. The 16srRNA primers were used for PCR analysis. **Results:** In humans, Ofloxacin and Ciprofloxacin were sensitive, ceftriaxone and ampicillin shows no sensitivity against bacteria. Cefixime shows resistance to both bacteria. In chicken and goat streptomycin was sensitive, while all others were not sensitive against bacteria. Azithromycin. Nalidixic acid, Pencillin and Ofloxacin were completely resistant. **Conclusion:** It is concluded that *E. Coli, P. aeruginosa, Enterobacter spp. and Klebsiella spp.* in humans and animals are first reported in division. These could be risk for human health because of the chance of transmission of these bacteria to people by food chain or direct interaction to humans.

Keywords: Antibiotics, humans, animals, bacteria, resistance

Introduction

Antimicrobial medications have been crucial in both animals and people to reduce infection and mortality that caused by contagious disorders (1). The main reason of developing and dissemination of drug resistance characteristics among pathogenic and mutualistic bacteria is the pressure due to usage of antibiotics (Aarestrup et al., 2008). Additionally, resistance has emerged after the

introduction of major class of antibiotics, with development times ranging from less than a year (penicillin) to more than ten years (vancomycin) (2).
Salmonella species and E.coli are Gram negative bacteria from the family of Enterobacteriaceae (3, 4). Escherichia. Coli are independent bacteria of animals and people. Infections in the digestive tract and beyond, such as cerebromeningitis, intestinal flu, UTI(urinary tract infections), peritoneal inflammation, and sepsis, are brought by pathogenic variations (5, 6). The majorities of E.coli stains are beneficial to active people and animal gastrointestinal tracts and are completely innocuous. Although, a few strains have developed virulence traits and are referred to as disease causing E. coli (3, 4). Depending on the infection type, different treatments are available. For instance, fluoroquinolones and trimethoprim/sulfamethoxazole has been recommended treatments for UTI's (7), while, antibiotic drug therapy is not advised for infections caused by Shiga toxin-producing Escherichia coli (8).
According to (9), P. aeruginosa isolates exhibit the widest range of resistance ratios based on strains root, which range from five percent to fifty percent (9-13) P. aeruginosa linked to the same issues as other nosocomial pathogen, including localized outbreaks, MDR strains, and spread in specific clinical sites like hospitals. The only antimicrobial drug that are taken orally, Fluoroquinolones, are frequently employed opposed to P. aeruginosa at dosages that are too low. Due to restrictions against the use of ciprofloxacin in few hospitals, some less effectual drugs that are most likely to choice resistant mutants are suggested instead.
Few published research have testified on antibiotic resistance in bacteria, mainly Salmonella and E. coli, despite the fact that many bacteria collected from chicken or chicken-related samples had been studied (14). Numerous research on mutualistic bacteria and medical isolates against antibiotics hane examined resistance in food animals like chicken, pigs, and cattle. (1, 15-26). In several of these studies, disease causing and commensal bacteria are found to be highly resistant to a range of medically important antibiotics (27). At the same time, little information has been published about the prominence of antibiotic resistance in small ruminants, and there is minimal global surveillance on this topic (28).
The majority of little ruminants raised on pasture, use little to no antibiotics, yet under grazing systems, these organisms are free to move around and interact with their surroundings. Additionally, it has been discovered that wild animals without antibiotic use contain antibiotic-resistant Escherichia coli (E. coli) in their systems (29, 30). Additionally, the digestive tract of little ruminants is home to several E. coli strains of public health importance, including those that are associated with the O157, O26, and O145 serotypes (31-33). Despite the fact that goats are an important source of meat and milk internationally, the appearance of their stomach E. coli strains have not been completely defined. The present study was conducted to isolate the *E. coli* and *P. aeruginosa* strains from poultry and goat samples, and *Enterobacter spp* and *Klebsiella spp.* of district Okara and determine the antibiotic resistance patterns against these bacteria. This study also characterizes the molecular pattern of the bacteria by PCR analysis.

Materials and Methods

Study area

This study was conducted in district Okara, Pakistan from 20 November 2021 to 20 February 2022. This study was carried out for the isolation, identification and detection of different bacteria from feces samples of typhoid animals and human patients, and detection of antibacterial susceptibility and resistance patterns in chicken, goats and humans.

Ethical statement

The study was conducted according to the declarations of Helsinki. Informed consent was signed from patients where applicable. The study on animals was according to Helsinki declarations. Consent to participate and consent to publication was signed from patients. Institutional or ethical review board certificate was obtained to complete the study.

Sample collection and placement

Fecal samples were collected randomly in different days and each farm was toured only one during this study period. Samples were collected by using sterile zipper bags, sterile urine containers and gloves. Two hundred twenty samples were collected from which forty samples were of goat, sixty of chicken and one hundred twenty of humans. 3 samples of humans, 2 samples of chicken and 1 sample of goat were positive for bacteria and further processed for identification of mutations. During collection of samples it was ensured that it was pure or uncontaminated sample. Another 1ml enriched broth was transferred to a 10ml and incubate at 37 C for 24 hours (34). The collected samples were transported to molecular biology lab in University Of Okara in one hour in ice box. The samples were stored at 4 C in the lab refrigerator for 24 hours.

Bacterial isolation

The isolates were cultured in nutrient broth for enrichment at 37˚C for 20 hrs (35). Every sample were grow on nutrient broth were sub cultured by streaking on Salmonella-Shiegella Agar (Becton–Dickinson Co., Sparks, MD, USA) for *E. coli* gram negative bacteria and incubated at 37° C for 16 to 24 hours. By using such a method all specimens were pre- enriched in buffered peptone water in the concentration as 1:10 and followed anaerobic incubation at 37° C for about 18 to 20 hours (34). Initially, a ten folded dilution of each sample was prepared in Eppendorf tubes by using 0.1% peptone water. Earlier for each specimen a plate count agar PCA was divided into different parts and each part marked separately. Dilutions were within the range of 10^{-1} to 10^{-8}. For each specimen 1ul was taken from each dilution and spread on each part of the PCA plate and were kept in incubator at 37° C for 24 h for the growth of bacteria as single colonies. After incubation period the colonies grows at different parts of PCA were counted from each dilution. The findings of the total bacterial colonies counted were mentioned as colony forming unit CFU/g or ml of the sample (34).

Biochemical tests

Different confirmation tests such as Gram staining and catalase tests were used to identify gram negative bacteria (36). Gram staining procedure was performed according to a system described by (37). The Catalase enzyme detoxify hydrogen peroxides by breaking it into gas and water, the bubbles formed from oxygen gas easily describe the Catalase test positive.

Antibiotic sensitivity pattern

The Antibiotic sensitivity test for every isolate was carried out according to the Kirby Bauer disc diffusion method. As it is recommended by National Committee Clinical Laboratory Standards NCCLS. This method is very successful for determination of effectiveness of different antibiotics by the measuring the diameter of zone of inhibition in growth of bacteria. Muller Hinton Agar was used as growth media for *E. coli* and for the inculation of antibiotics to determine the zone of inhibition (38). In this method a suspension or colonies of the organism were streaked on to artificial media Muller Hinton Agar and different paper discs containing different concentration antibiotic agents applied on streaked plates. The antibiotic containing plates then incubated in incubator for 16 to 18 hours at temperature 35° C. After incubation period, zone of inhibition was noted by measuring the circle around the paper antibiotics, the circles around antibiotics where the bacterial organisms do not grow (39). The zone of inhibition around the disks was used to classify the bacteria as susceptible, resistant or intermediate according to CLSI criteria (40).

Antibiotic discs used against bacteria

A total of 13 antibiotic discs (Becton Dickinson, U.S.A.) with Chloramphenicol (C 30µg/disc), Tetracycline (TE 30µg/disc), Penicillin (P 10 µg/disc), Neomycin (N 30µg/disc) (34), Azithromycin (AZM 30 ug/disc), Cefixime (CFM 5 ug/ disc), Ciprofloxacin (CIP 5ug/disc), Nalidixic Acid (NA 30ug/ disc) (41), Ampicillin (AMP 10 µg/ml/disc), gentamycin (GN 10 µg/disc) (42) Streptomycin

(STR 10 µg/ml/disc, Ceftriazone (CRO 30ug/disc), Cefoxitin (FOX 30ug/disc) (43-45) were used for determination effectiveness against bacteria.
Five antibiotics were used on all human patients sample for resistance and susceptibility pattern. In patients, antibiotic discs Ceftriaxone CRO 30ug/disc, Ampicillin AMP 10ug/disc, Ciprofloxacin CIP 5 ug/disc, and Ofloxacin OFX 5ug/disc, Cefixime CFM 5ug/disc, were used to check the resistance and susceptibility patterns (46-48).

DNA Extraction and quantification
Phenol chloroform natural process was employed for the extraction of DNA (49). The DNA was extracted according to the protocol followed by (50). The quality and concentration of DNA was measured by Nanodrop plate method (Skanit RE 4.1, Thermoscientific). Absorbance at 260, 280 and 320 nm were calculated. The 260/280 ratio ranges from 1.3 to 1.9 which confirms the good quality DNA where the DNA concentration was approximately 625 to 2100 ng/µL.

Polymerase Chain Reaction (PCR)
Chemical with fixed quantity have been employed for Template DNA including forward and reverse Primers (Macrogen Company), Taq polymerse enzyme 5U/ µL (Thermo scientific 01047431), PCR buffer (Thermo scientific 01047431), magnesium chloride (Thermo scientific 01047431), dNTPs (Thermo Scientific 01044193) and PCR water (Invitrogen RT PCR grade water, AM9935)
A single copy of DNA or a particular DNA sequences can be enlarged by molecular biology procedure known as PCR (51). The amplified bacterial DNA was produced using 1492/27 primer barcodes. Forward and reverse primer sequences includes: reverse primer was 1492R: GGTTACCTTGTTACGACTT and forward primer was 27F \ 8F: AGAGTTTGATCCTGGCTCA. On a Galaxy XP thermal cycler (BIOER, PRC), PCR was performed. The whole process of PCR was employed used by (52).

PCR Gel Electrophoresis Analysis:
A product that had been amplified was run on a 2 percent agarose gel and seen under a UV light. The amplified samples were run against the 100 bp DNA ladder PLUS (Thermo scientific), which is shown in the representative PCR images below. The obtained product size was greater than 1200 bp. The human samples in figure 8 were H.T. 1.1, H.T. 2.1, and H.T. 4.1 and 4.2.

16S rRNA gene sequence, BLASTn and phylogenetic analysis
Identification of strains was completed by 16S rRNA sequences study by sequencer, obtained sequences were related with sequences already present in GeneBank, obtained sequences align by Basic Local Alignment Search Tool (BLAST) tool, the high S-ab values with recognized species in Sequence match research. Following the outcomes established by BLASTn the evolutionary tree was generated by BLASTn (52).

Statistical analysis
Collected information was added to MS excel– 2010. Descriptive analysis was performed and shows as percentage of various variables including resistance, intermediate and sensitivity patterns of antibiotics (53).

Results
During this study, the total of 100 samples from animals including 60 samples from chicken and 40 samples from goat was collected from which the bacteria *E.coli* and *P. aeruginos,* obtained while total of 120 samples were collected from humans from which *Enterobacter and Kllebsiella spp.* strains obtained from fecal samples.

Among the *E.coli* and *P. aeruginosa, Enterobacter and Kllebsiella spp.* strains obtained from fecal samples of chicken, goat and humans all samples were subjected to biochemical tests by gram staining and catalase test. The *E. coli* was found in 15 sample of chicken constitutes 25% and *P. aeruginosa* was also found in 15 sample of chicken also constitutes 25% of the total 60 samples. The *E. coli* was found in 10 sample of goat from 40 samples constitutes 25%. The *Enterobacter spp.* was found in 10 samples constitutes 8.33% while the *Kllebsiella spp.* was found in 20 samples constitutes 16.67% from 120 patient's samples. The percentage of positive bacteria in chicken, goat and humans is shown in table 1. All remaining samples were negative for any bacteria.

Sample specie	**Sample source**	**No. of samples**	**E. coli positive samples**	**% of E. coli positive samples**	**P. aeruginosa positive samples**	**% of P. aeruginosa positive samples**
Chicken	Fecal samples	60	15	25.00	15	25.00
Goat	Fecal samples	40	10	25.00	0	0.00
Total		100				
Sample specie	**Sample source**	**No. of samples**	***Enterobacter spp.* positive samples**	**% of *Enterobacter spp.* positive samples**	***Klebsiella spp.*positive samples**	**% of *Klebsiella spp.* positive samples**
Humans	Fecal samples	120	10	8.33	20	16.67
Total		120				

Table 1: Shows the no. of positive gram negative bacteria

Antibiotic sensitivity patterns

In chicken and goat, 13 antibiotics were used and 5 antibiotics were used in humans. In chicken and goat streptomycin was completely sensitive to the *E. Coli* bacteria, tetracycline shows 86.67% intermediate and 13.3% shows sensitivity, Ceftriaxone shows 86.67% resistance and 13.3% shows sensitivity, Ampicillin shows 86.67% sensitivity and 13.3% shows intermediate. Azithromycin. Nalidixic acid, Pencillin G and Ofloxacin shows 100% resistance. Cefixime shows 86.67% resistance and 20% shows intermediate, Ciprofloxacin shows 100% sensitivity to the bacteria, Cefoxitin shows 60% resistance and 13.3% sensitivity. The Neomycin and Gentamicin shows 86.67% sensitivity and 13.3% shows intermediate results. Chloramphenicol shows 13.3% sensitivity and 86.67% resistance. This is shown in table 2. In chicken streptomycin was completely sensitive to the *P. aeruginosa* bacteria, tetracycline and Ceftriaxone shows 86.67% sensitivity and 13.3% was intermediate to tetracycline and 13.3% was resistant to Ceftriaxone, Ampicillin shows 6.667% sensitivity and 93.3% shows intermediate. Azithromycin shows 100% resistance. Cefixime shows 93.3% resistance and 6.667% shows resistance, Ofloxacin and Ciprofloxacin shows 100% sensitivity to the bacteria, Cefoxitin shows 13.33% resistance and 86.67% sensitivity. The Neomycin shows 80% sensitivity and 20% shows intermediate and Gentamicin shows 13.3% sensitivity and 86.67% shows intermediate results. Chloramphenicol shows 20% sensitivity and 80% resistance. *P. aeruginosa* bacteria was not observed in goat. This is shown in table 3.

In humans, 5 antibiotics were used against isolated bacteria. Ofloxacin and Ciprofloxacin was completely 100% sensitive to the Enterobacter spp., Ceftriaxone and Ampicillin shows 60% resistance and 20% shows sensitivity and intermediate. Cefixime shows 100% resistance to the bacteria. Ofloxacin and Ciprofloxacin was completely 100% sensitive to the Klebsiella spp., Ceftriaxone and Ampicillin shows 60% resistance and 20% shows sensitivity and intermediate. Cefixime shows 100% resistance to the bacteria. This is shown in table 4.

Antibiotic susceptibility pattern of strains of Escherichia coli														
Antibiotic Discs	**Symbol**		**Sensitivity Groups of bacterial Isolates from chicken and Goat**											
			Resistant				**Intermediate**				**Sensitive**			
		no. of samples	**no. of strains positive**	**% of strains positive**	**Inhibition zone (mm)**	**no. of samples**	**no. of strains positive**	**% of strains positive**	**Inhibition zone (mm)**	**no. of samples**	**no. of strains positive**	**% of strains positive**	**Inhibition zone (mm)**	
Streptomycin	**S**	15	0	0	≤6	15	0	0	7-9	15	15	100	≥10	
Tetracycline	**TE**	15	0	0	≤14	15	2	13.3	15-18	15	13	86.67	≥19	
Ceftriazone	**CRO**	15	13	86.67	≤19	15	0	0	20-22	15	2	13.33	≥23	
Ampicillin	**AMP**	15	0	0	≤13	15	2	13.3	14-16	15	13	86.67	≥17	
Azithromycin	**AZM**	15	15	100	≤13	15	0	0	14-17	15	0	0	≥18	
nalidixic acid	**NA**	15	15	100	≤ 13	15	0	0	14-18	15	0	0	≥19	
Cefixime	**CFM**	15	13	86.67	≤15	15	0	0	16-18	15	2	13.33	≥19	
Ciprofloxacin	**CIP**	15	0	0	≤15	15	0	0	16-20	15	15	100	≥21	
Cefoxitin	**FOX**	15	11	73.33	≤14	15	0	0	15-17	15	4	26.67	≥18	
Neomycin	**N**	15	0	0	≤12	15	2	13.3	13-16	15	13	86.67	≥17	
Gentamicin	**CN**	15	0	0	≤12	15	2	13.3	13-14	15	13	86.67	≥15	
Chloramphenicol	**C**	15	13	86.67	≤12	15	0	0	13-17	15	2	13.33	≥18	
Pencillin G	**P**	15	15	100	≤15	15	0	0	13-17	15	0	0	≥15	
Ofloxacin	**OFX**	10	0	0	≤12	10	0	0	13-15	10	10	100	≥16	

Table 2: Antibiotics susceptibility patterns of strains of *E. coli*

Antibiotic susceptibility pattern of strains of *P. aeruginosa*													
Antibiotic Discs	**Symbol**		**Sensitivity Groups of bacterial Isolates from chicken and Goat**										
			Resistant				**Intermediate**				**Sensitive**		
		no. of samples	**no. of strains positive**	**% of strains positive**	**Inhibition zone (mm)**	**no. of samples**	**no. of strains positive**	**% of strains positive**	**Inhibition zone (mm)**	**no. of samples**	**no. of strains positive**	**% of strains positive**	**Inhibition zone (mm)**
Streptomycin	**S**	15	0	0	≤6	15	0	0	7--9	15	15	100	≥10
Tetracycline	**TE**	15	0	0	≤14	15	2	13.3	15-18	15	13	86.67	≥19
Ceftriazone	**CRO**	15	2	13.33	≤19	15	0	0	20-22	15	13	86.67	≥23
Ampicillin	**AMP**	15	0	0	≤13	15	14	93.3	14-16	15	1	6.667	≥17
Azithromycin	**AZM**	15	15	100	≤13	15	0	0	14-17	15	0	0	≥18
nalidixic acid	**NA**	15	0	0	≤ 13	15	15	100	14-18	15	0	0	≥19
Cefixime	**CFM**	15	1	6.667	≤15	15	14	93.3	16-18	15	0	0	≥19
Ciprofloxacin	**CIP**	15	0	0	≤15	15	0	0	16-20	15	15	100	≥21
Cefoxitin	**FOX**	15	2	13.33	≤14	15	0	0	15-17	15	13	86.67	≥18
Neomycin	**N**	15	0	0	≤12	15	3	20	13-16	15	12	80	≥17
Gentamicin	**CN**	15	0	0	≤12	15	13	86.7	13-14	15	2	13.33	≥15
Chloramphenicol	**C**	15	12	80	≤12	15	0	0	13-17	15	3	20	≥18
Pencillin G	**P**	15	15	100	≤15	15	0	0	13-17	15	0	0	≥15
Ofloxacin	**OFX**	10	0	0	≤12	10	0	0	13-15	10	10	100	≥16

Table 3: Antibiotics susceptibility patterns of strains of *P. aeruginosa*

Antibiotic susceptibility pattern of strains of Enterobacter spp. and Klebsiella spp.													
Antibiotic Discs	**Symbol**		**Sensitivity Groups of bacterial Isolates from Humans**										
			Resistant				**Intermediate**				**Sensitive**		
		no. of samples	**no. of strains positive**	**% of strains positive**	**Inhibition zone (mm)**	**no. of samples**	**no. of strains positive**	**% of strains positive**	**Inhibition zone (mm)**	**no. of samples**	**no. of strains positive**	**% of strains positive**	**Inhibition zone (mm)**
Enterobacter spp.													
Ofloxacin	**OFX**	10	0	0	≤12	10	0	0	13-15	10	120	1200.00	≥16
Cefixime	**CFM**	10	10	100	≤15	10	0	0	16-18	10	0	0.00	≥19
Ceftriaxone	**CRO**	10	6	60.00	≤19	10	2	20	20-22	10	2	20.00	≥23
Ampicillin	**AMP**	10	6	60.00	≤13	10	3	30.00	14-16	10	1	10.00	≥17
Ciprofloxacin	**CIP**	10	0	0	≤15	10	0	0	16-20	10	10	100.00	≥21
Klebsiella spp.													
Ofloxacin	**OFX**	20	0	0.00	≤12	20	0	0.00	13-15	20	20	100.00	≥16
Cefixime	**CFM**	20	20	100.00	≤15	20	0	0.00	16-18	20	0	0.00	≥19
Ceftriaxone	**CRO**	20	12	60.00	≤19	20	4	20.00	20-22	20	4	20.00	≥23
Ampicillin	**AMP**	20	12	60.00	≤13	20	4	20.00	14-16	20	4	20.00	≥17
Ciprofloxacin	**CIP**	20	0	0.00	≤15	20	0	0.00	16-20	20	20	100.00	≥21

Table. 4: Antibiotic susceptibility pattern of strains of Enterobacter spp. and Klebsiella spp.

Agarose Gel Electrophoresis

Figure 1 gel picture shows descriptive DNA bands in relation to 1KB Ladder. In figure 1, H.T 1.1, H.T 2.1 and H.T 4.1 and 4.2 were the samples of humans, G1.1, G1.2, G2.2, G2.2 represents goat, Chk 1.1, Chk 1.2, Chk 2.1, Chk 2.2, Chk 3.1, Chk 3.2, Chk 4.1, Chk 4.2, Chk 5.1 and Chk 5.2 represents chicken.

Figure 1: Shows that 1KB ladder was loaded in wells with DNA samples

PCR Gel Electrophoresis Analysis:

Figure 2 of PCR, 100 bp DNA ladder PLUS was run beside enlarged isolates. Greater than 1200 bp product size was obtained. In figure 2, H.T 1.1, H.T 2.1 and H.T 4.1 and 4.2 were the samples of humans, G1.1, G1.2, G2.1, G2.2 represents goat, Chk 1.1, Chk 1.2, Chk 2.1, Chk 2.2, Chk 3.1, Chk 3.2, Chk 4.1, Chk 4.2, Chk 5.1 and Chk 5.2 represents chicken.

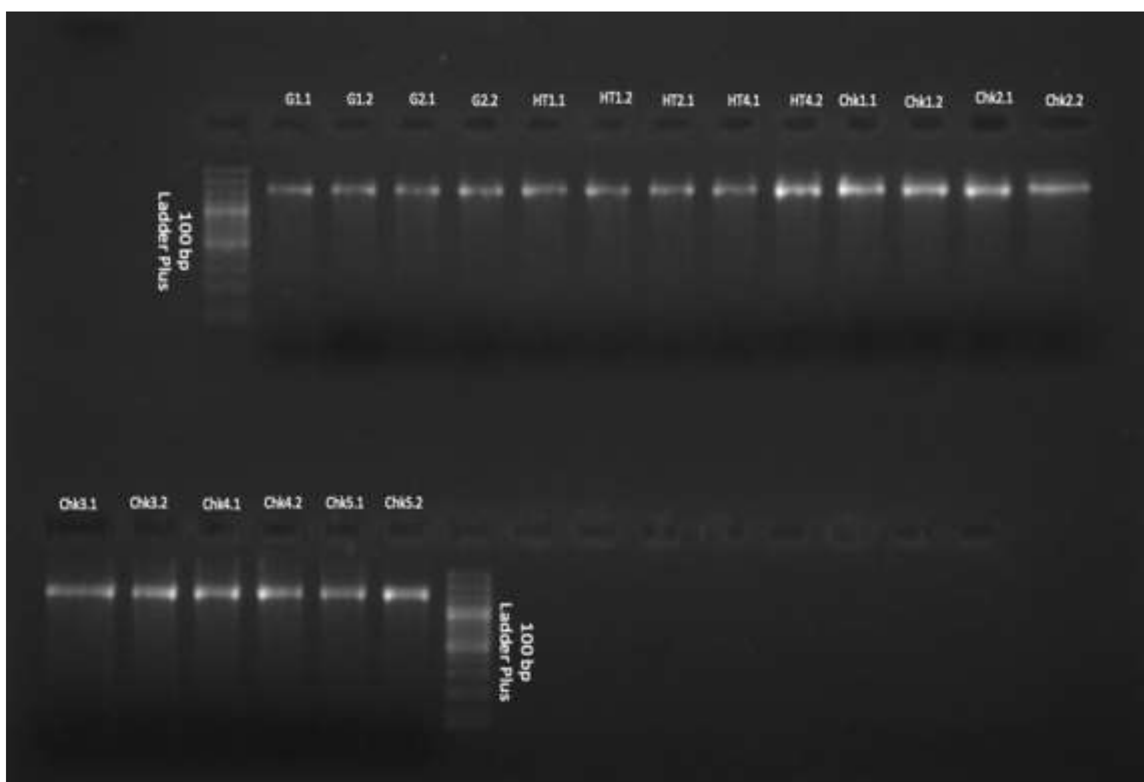

Figure 2: Shows PCR Gel Electrophoresis Analysis

Phylogenetic Tree Based on the 16S rRNA gene Sequences

The phylogenetic study was done on the protein sequences of new strains identified in the region and were obtained from BLAST outcomes and employed as input for evolutionary tree construction. The results of sequences were aligned and evolutionary tree was reconstructed and is shown in Figure 3. The clustering patterns show the genetic and phylogenetic relationships in bacterial species. Two clusters were generated. The *Klebsiella sp*., *Enterobacter sp*. and *Escherichia coli* was in 1 cluster and *P. aeruginosa* was in 2nd cluster

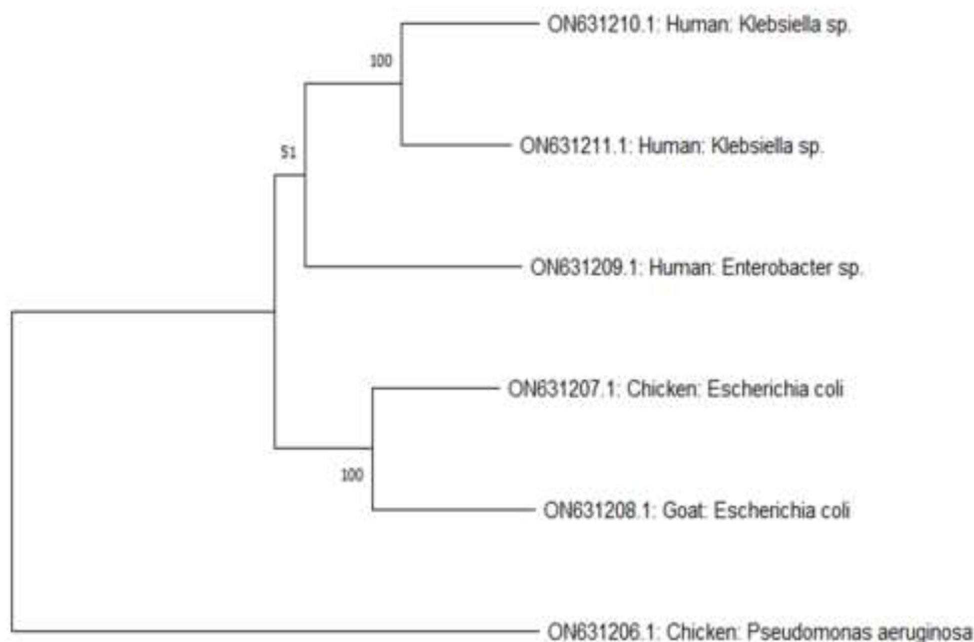

Figure 3: Phylogenetic tree shows the resemblance of 16S rRNA sequences

Nucleotide sequences and Accession number

The nucleotide sequences were submitted in the ncbi website (https://www.ncbi.nlm.nih.gov/). The obtained accession number includes ON631206 of *Pseudomonas aurignosa,* ON631207 of *E. Coli* from chicken and ON631208 of *E. Coli* from goat. The accession number of humans includes ON631209 of *Enterobacter spp.* and ON631210, ON631211 of *Klebsiella spp.*

Discussion

The results of the present study investigated that *K. pneumonia* (53.3%) is dominated over *Escherichia coli* (13.3%) and *Staphylococcus* species. These findings can be compared well with the outcomes of (54) who investigated that *Klebisella species* is more common than *Escherichia coli* in urinary tract infections. These outcomes contradictory to the work of others who investigated *Escherichia coli* to be more dominated over *Klebisella spp*. on UTIs studies (54, 55) and (56) who described *S. epidermidis* (22) to be more dominant pathogens over *Staphylococcus aureus* (16) *Streptococcus spp.* (5), *Escherichia* coli (4) and *K. pneumonia* (3) and two samples of *Proteus* species*, En. aerogenes* and *B. cereus* in corresponding studies on experimental specimen. Other pathogens identified in order of commonness involve *P. aeruginosa*, *S. aureus and S. pyrogenes* but the 53.3% noted for *Klebisella* species although revealed that the *klebisella* species are acquiring more importance as UTI etiologic means than formerly indicated by many of the other investigators (55, 57-59). In this study, the *Enterobacter and Kllebsiella spp.* strains obtained from fecal samples

of humans. The *Enterobacter spp.* was found in 10 samples out of 120 samples constitutes 8.33% while the *Kllebsiella spp.* was found in 20 samples out of 120 samples constitutes 16.67%
Klebsiella spp. (53.3%), *P. aeruginosa* (20.0%), *E. coli* (13.3%), *S. aureus* (6.7%) and *S. pyrogenes* (6.7%) were the prevailing pathogens identified in these clinical samples. This result vary from other studies which declare that Gam-negative bacteria, mostly *Escherichia coli* is the most intimating pathogen identified in patients clinical samples from UTIs patients (60, 61) and other studies which also reported Gram positive bacteria, especially *S. epidermidis* to be the most familiar and dominated pathogen identified in other experimental specimens (56).
Through antibiotic sensitivity tests, it was indicated that all the pathogens of bacteria were equally sensitive to Ofloxacin and Ciprofloxacin in magnitude. These were also susceptible to few other antibiotics with *E. coli* with greatest sensitivity to Nitrofurantoin (100 %) as contrary to (61) who declared decreased sensitivity of *Escherichia coli* to this was noted only 63.6% of the *Escherichia. coli* was susceptible to drugs. The susceptibility to Peflacin, Nitrofurantoin, Gentamicin, Streptomycin and Cotrimoxazole were 55.3%, 33.3%, 6.7% and 6.7% respectively. It was related to the studies of (60) who revealed susceptibility to ofloxacin (71.3%), (61) who also described 97.1% susceptibility to Ofloxacin, 77.8% to Ciproflaxin and 50% to Nitrofurantoin. This is divergence from the study of (56) who indicated the susceptibility of Gram negative samples to Peflacin, Gentamycin, Streptomycin and Ciproflox to be 100%, 100, 90.9 and 63.6% respectively and Gram positive isolates to be 93.3 and 82.2% sensitivity to Gentamycin and Streptomycin respectively. For treatment of UTI, antibiotics including CIP, Ofloxacin and Nitrofurantoin can be suggested when cure is needed. A total of 5 antibiotics were used in this study against identified bacteria. Ofloxacin and Ciprofloxacin was completely 100% sensitive to the Enterobacter spp., Ceftriaxone and Ampicillin shows 60% resistance and 20% shows sensitivity and intermediate. Cefixime shows 100% resistance to the bacteria. Ofloxacin and Ciprofloxacin was completely 100% sensitive to the Klebsiella spp., Ceftriaxone and Ampicillin shows 60% resistance and 20% shows sensitivity and intermediate. Cefixime shows 100% resistance to the bacteria.
The Gram- negative isolates showed high resistance to AMP, C and TET followed by Cotrimoxazole, G, Nitrofurantoin and S. Antibiotics resistance in the Enterobacteriaceae *(Klebsiella pneumoniae, Escherichia coli)* was common for these drugs, *Escherichia coli* considered to be resistant to these drugs. This accepts the results of (56). This was favorably reported that Gram negative bacilli foster series of antibiotics resistance genes which can be transmitted to other bacteria smoothly (62-65). All bacilli identified such as *Escherichia. coli, K. pneumoniae* and *P. aeruginosa* have been appeared to cause distinct nosocomial infection (56, 64-69). In this study, isolated Gram- negative *P. aeruginosa* isolates showed 100% resistance to 5 from 10 antibiotics in vitro (AMP, C, Cotrimoxazole, Nitrofuratoin and TET), 66.7% to G and S and 33.3% to Peflacin, but 100% sensitive to Ofloxacin and CIP and 66.7% sensitive to Peflacin. Multi drug resistanct P. aeruginosa was also identified by (70) in the study of nosocomial infection of UTIs and (71) on experimental specimens. (61) identified multi drug resistant *P. aeruginosa* to regulate the prevalence of UTIs in children and teenagers.
They observed that MDR was common (50.0%) and increasing by 75% during 2002 to 2007. MDR in the US among 38,835 UTI samples was 7.1% in 2000 (72). MDR has significant consequences for empiric treatment of disease affected by *K. spp., P. aeruginosa, Escherichia coli, Staphylococcus aureus* and *S. pyrogenes* and for the variety of antimicrobial resistance facilitated by MDR plasmids (73, 74). These consequences are not astonishing. Early research had now touched the range of difficulty in Nigeria (56, 60, 61, 70). Same information have also been described from other states in Australia (75, 76) Europe (76, 77), Asia Pacific and Singapore (75, 76, 78-80). By comparing with same information from Europe (76, 77) and Australia (75, 76) , frequency of resistance in gram-negative bacteria is very high. The reason for the changes in MDR pattern may be associated to disease control management or to control the resistant bacteria. Conversely, more study is required to explain these variances.

They revealed the highest resistance proportions of isolates of *Escherichia coli* and *Klebsiella sp*. to frequently used drugs, including streptomycin (98%), cloxacillin and oxacillin (96%), and colistin (84%), with comparatively low resistance ratio to CIP (66%); this was same like those from neighbor states, Kenya (81) and Uganda (82). These results can probably results from the relation of samples with AmpC enzymes. *Esccherichia coli* revealed the high resistance towards streptomycin, also cloxacillin, oxacillin, and erythromycin shows resistance. However, the experimental samples were susceptible to amikacin (36%), and also to ofloxacin (26%), cefotaxime (18%), G, and CIP (22%). The sensitivity was same detected by (83). *Klebsiella* were highly resistant towards cefotaxime (93.75%), and also to cloxacillin, oxacillin, and colistin sulfate (91.67%). A significant resistance occurs with streptomycin, like in *E. coli* and also shown by (84). They observed highest susceptibility ratio towards amikacin (33.37%), like observed in *E. coli*, and also to gentamycin and cefepime (22.92%). They also displayed high MDR occurs in *Escherichia coli* with MAR index of 1.00, and 17 out of 50 samples (34%) shows resistance to fourteen antibiotics. The MAR index of *Klebsiella sp.* was 0.93, which varies from other research (85) that described an index of 0.4; in contrast to the low resistance to aminoglycosides described in early studies, They described resistance of 87.5%, 68.75%, and 58.38% in S, GEN, and amikacin antibiotics respectively.
For *Klebsiella sp*., seven isolates (14.6%) shows resistance to fourteen antibiotics, however 12 samples (25%) shows resistance to thirteen out of fourteen discs used. This was same to the work of (85) where 66.7% of the *Klebsiella* samples completed a MAR index more than 0.2. The *Escherichia. coli* was susceptible to fluoroquinolone about 80%, and these result relates with previous work (86) that described an average of 80% susceptibility in various regions. In (87) *Klebsiella* shows resistance to ampicillin and 3rd generation cephalosporin, which verifies the results of this study. In this study, the *Kllebsiella spp.* was found in 20 samples from total 120 samples and shows resistance to Cefixime. Ofloxacin and Ciprofloxacin were completely sensitive; Ceftriaxone and Ampicillin were 60% resistant and 20% shows sensitivity and intermediate.
The 235 samples of *E. coli* of cattle, pig and chicken were examined for the resistance to 7 antibiotics by disc diffusion technique. Minimum inhibition range was obtained for 154 samples shows resistant to at least 1 of the antibiotics used. Resistance was observed in 65.5% and MDR to two antibiotics in 37.9% of samples. Resistance was high in *E. Coli* bacteria from chicken (74.0%), also in pig (64.8%) and cattle (61.3%). The frequent resistance to ampicillin, S, TET, sulphamethoxazole/trimethoprim, and KAN i.e., 42.5-11.9% was observed. Resistance to KAN, sulphamethoxazole/trimethoprim, and TET was low in cattle (2.5-7.5%) than other animals i.e., 12.0-40.0% and ($p < 0.01$). Streptomycin and AMP were high resistant respectively ($p < 0.01$). Same resistance ratio was seen in fecal samples (29.9%) and carcass swab (33.1%) samples. 40 resistant forms were noted from which only 5 (12.5%) were frequently occurs in the samples under study (88). This work showed that the *Escherichia. coli* was found in 15 samples of chicken constitutes 25% and *P. aeruginosa* was also found in 15 sample of chicken also constitutes 25% of the total 60 samples.
They found that E. coli samples shows resistance in goats offspring's on meadow as early as 3 week of age in the deficiency of use of antimicrobials in goat children study and the treatment does for the early 1 year. However, these were novel results not early observed in goats, others described the early colonization by resistance to *Escherichia coli* in young organisms of other classes even earlier contact to precise antimicrobials or soon after management (89, 90). Moreover, antibiotics resistant samples were also described in untouched ecosystem that were not visible to antibiotics in early periods (29, 90), which proposes that many aspects may be answerable for antibiotics resistance achievement and spreads in the surrounding. In some studies, aspects are not related with direct antibiotics use, containing environmental position, housing, animals age, and determination of production, were also observed to have an influence on the occurrence of *Escherichia coli* resistant to antibiotics (15, 17). They found high ratio of resistant E. coli samples were noticed in young goat children than in treatment does in same surroundings through the pre-weaning time. Frequency of resistance in goat's children was highest but reduced in post weaning, getting low level by 1 year of

age. Their results were same like that of (17, 89, 91-93) who identified high colonization by *Escherichia coli* resistance in young babies and pigs that failed with age of organisms. Their results were same of Berge et al 2010 results in cattle who described a high frequency of antibiotic resistance in young organisms than old species and also earlier colonization samples showing resistance in the absence of use of antibiotics. They observed, while many samples found organisms with 3 weeks of age shows intermediate resistance, this altered with time and by the time the animals were 3 months and yonder, most resistant *E. coli* shows full resistant phenotype. It is not clear that why this occurs though their hypothesis is animal combining with bacterial aspects might plays a part in less existence of samples with intermediate resistance in old hosts. To their information, this was not described in other research (94). This work comprises of the *E. coli* was found in 10 sample of goat from 40 samples constitutes 25%.

In *E.coli*, a large number of antimicrobial resistance was found either individually or combined against to a variety of antibiotics in this study's goat sample. Tetracycline, streptomycin, gentamicin, chloramphenicol, amoxicillin, nalidixic acid, tobramycin, ampicillin and were among the drugs for which resistance was found. According to other research in food animals, tetracycline, streptomycin, ampicillin, and amoxicillin/clavulanic acid were the antibiotics most commonly resistant to, with little resistance to other antibiotics. In food animals, the most frequently found antibiotics are tetracycline and streptomycin, for the reason that these drugs were available in the shops for many years and used in food animals. Although it had been more than a year prior to the start of the investigation, tetracycline had previously been used on the farm for cure in the recent study. It's likely that tetracycline-resistant bacterium isolates were present or continued to be in soil or in intestines of some organisms before colonizing the study animals while they were grazing. According to research, once bacteria start antibiotic resistance, this phenotype can still be seen up to 4 years after the last antibiotic usage (95, 96). Many studies revealed that genes for tetracycline can survive for a long time in the soil where it has been modified or blended with animal dung (19, 97). In this research, a few aged animals that had been housed on the location for more than a year included isolates that were tetracycline-persistent. Despite no prior usage of this antibiotic on the farmhouse, a large percentage of *Escherichia coli* samples in this investigation showed resistance to -lactams, particularly ampicillin. This suggests that this particular resistance can have originated from other environmental factors independent to farming operations. Our findings are consistent with those of earlier studies showing ampicillin and other antibiotic resistance in feral animals unassociated to known antibiotic use (30).

They found that, 1 sample was resistant to 4 antibiotics, but the majority of samples were only resistant to 1 or 2 antibiotics. Given that no antibiotics were administered to the cohort during the study duration and the fact that these animals were primarily raised on pasture, this is a remarkable conclusion. These organisms can carry resistant isolates that can travel long distances via flow and overspill of water. In the United States, studies on cattle, swine, and poultry similarly reveal a significant percentage of isolates that are resistant to multiple antibiotics (1). However, in the US, these animals are raised in severe production methods, which have historically been linked to substantially peak level of antibiotics use. In this study, the total of 13 antibiotics was used against bacteria in chicken and goat. . In chicken and goat streptomycin was completely sensitive to the *E. Coli* bacteria, tetracycline shows 86.67% intermediate and 13.3% shows sensitivity, Ceftriaxone shows 86.67% resistance and 13.3% shows sensitivity, Ampicillin shows 86.67% sensitivity and 13.3% shows intermediate. Azithromycin. Nalidixic acid, Pencillin G and Ofloxacin shows 100% resistance. Cefixime shows 86.67% resistance and 20% shows intermediate, Ciprofloxacin shows 100% sensitivity to the bacteria, Cefoxitin shows 60% resistance and 13.3% sensitivity. The Neomycin and Gentamicin shows 86.67% sensitivity and 13.3% shows intermediate results. Chloramphenicol shows 13.3% sensitivity and 86.67% resistance. This is shown in table 2. In chicken streptomycin was completely sensitive to the *P. aeruginosa* bacteria, tetracycline and Ceftriaxone shows 86.67% sensitivity and 13.3% was intermediate to tetracycline and 13.3% was resistant to Ceftriaxone, Ampicillin shows 6.667% sensitivity and 93.3% shows intermediate.

Azithromycin shows 100% resistance. Cefixime shows 93.3% resistance and 6.667% shows resistance, Ofloxacin and Ciprofloxacin shows 100% sensitivity to the bacteria, Cefoxitin shows 13.33% resistance and 86.67% sensitivity. The Neomycin shows 80% sensitivity and 20% shows intermediate and Gentamicin shows 13.3% sensitivity and 86.67% shows intermediate results. Chloramphenicol shows 20% sensitivity and 80% resistance. *P. aeruginosa* bacteria was not observed in goat.

Additionally, the use of molecular methods elaborates the causes of infectious diseases and help in antibiotic therapy (Clarridge III, 2004; Woo et al., 2008). The molecular mass of the natural DNA of the sequenced bacteria was determined by PCR to be 1500bp. MDR samples exhibited more than eighty percent resemblance in the NCBI GenBank by BLASTn, in accordance with the 16S rDNA studies. According to the BLASTn outcomes, the samples identified are *S,enterica subsp. enterica serovar Typhi str.* CT18, *Proteus mirabilis str.* HI4320, *Pseudomonas fluorescens str.* SBW25, *Staphylococcus cohnii subsp. cohnii str.* 532 Contig16, *Salmonella enterica subsp. enterica serovar Infantis, E.coli* strain*, K. pneumonia str.* J1, K12 *sub-strain*, *Shigella flexneri* 2a strain, DH10B. The outcome also showed that *Staphylococcus cohnii subsp. cohnii str*-532 Contig16, *Salmonella enterica subsp. enterica serovar Infantis*, and *Pseudomonas fluorescens* SBW25 had different cultural identifications. This was also mentioned via (98), who noted variations between the traditional method and the molecular method of identifying bacteria. The combination of conventional methods and molecular method will increase bacteriological analysis and permit for the definite and effective recognition of disease causing bacteria, which will help save human lives. Though, the outcomes of this work clearly show the interest in and viability of introducing the 16S rDNA gene sequencing process in Akure for the identification of human disease causing bacteria of fowl source (99). In this study, the 16SrRNA primers were employed to study the resistance patterns. The DNA was extracted and PCR was done. The quantification of DNA was done and the agarose gel electrophoresis was performed. The strain obtained from chicken during the study was highly relevant to *Pseudomonas aurignosa* and *Escherichia. Coli* and after submission to the ncbi the accession number of the strains is ON631206 and ON631207 respectively. The strain obtained from goat during the study was highly relevant to *Escherichia. Coli* and after submission to the ncbi the accession number of the strain is ON631208. The strain obtained from humans during the study was highly relevant to *Enterobacter spp* and *Klebsiella spp.* and after submission to the ncbi the accession number of the strain is ON631210 and ON631211 respectively.

Conclusion

It is concluded that all bacteria including *E. Coli, P. aeruginosa, Enterobacter spp. and Klebsiella spp.* in humans and animals are first reported in division sahiwal. Different antibiotics were used against bacteria for humans. For humans Ofloxacin and Ciprofloxacin are effective for treatment while ceftriaxone, ampicillin and cefixime are less effective for cure. In chicken and goat streptomycin is effective while tetracycline, Ceftriaxone, Ampicillin, Azithromycin and Nalidixic acid, Pencillin G, Cefixime, Ciprofloxacin, Cefoxitin, Neomycin, Gentamicin, Chloramphenicol are less effective for the treatment of *P. aeruginosa* in chicken and *E. Coli* in goat. These bacteria could be risk for human's health because of the chance of transmission of these bacteria to people by food chain or direct interaction of mobile resistant elements to humans.

Statements & Declarations

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Competing interest

Authors declare not any competing interest.

Author's contribution

All authors contributed equally in the work.

Data availability
Data will be available on demand of the authors.
Ethics approval
Ethics approval was obtained from university.
Consent to participate
Consent form was signed for data collection on human samples and permission was obtained from animal's manager.
Consent to publish
Consent was signed for publication.
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