emergent
Life Sciences Research

Research Article

Antibiotic susceptibility patterns of pathogenic bacteria recovered from unprocessed bovine milk produced in Ndivisi Ward, Bungoma County

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Received: 15 July 2020
Accepted: 24 October 2020
Online: 24 April 2021

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Emer Life Sci Res (2021) 7(1): 28-35

E-ISSN: 2395-6658
P-ISSN: 2395-664X

DOI: https://doi.org/10.31783/elsr.2021.712835

Abstract

This study was conducted to determine the antibiotic susceptibility patterns of pathogenic bacteria recovered from unprocessed bovine milk. Bacterial communities were isolated from the milk samples and then subjected to antibiotic susceptibility testing. Pure colonies were used to avoid contamination. The study was carried out in Ndivisi ward, Bungoma County, Kenya, between October 2016 to January 2017. The level of antibiotic resistance among the isolates was tested to amoxicillin, chloramphenicol, kanamycin, gentamicin, cephalexin, and tetracycline. The responses of the isolates to antibiotics were determined by measuring the diameter of the zone of inhibition around the antibiotic disk. These measurements were subsequently converted into a qualitative scale using the standard charts. Data on the bacteriological quality of milk were summarized using means. Means were determined since each bacterium species had several isolates. The percentages of bacteria resistant to antibiotics included amoxicillin (63%), kanamycin (19%), cephalexin (41%), and tetracycline (19%). Intermediate ones were kanamycin (33%) and cephalexin (22%). Susceptible ones were amoxicillin (37%), gentamicin (100%), kanamycin (48%), cephalexin (37%), chloramphenicol (100%) and tetracycline (81%). Generally, 62% of the bacteria were resistant, 33% were intermediate while 5% were susceptible. *B. subtilis*, *P. aeruginosa*, and *C. freundii* were multidrug-resistant bacteria. Cephalexin and kanamycin were intermediate to *E. coli* and *B. subtilis*. *K. pnemoniae* and *S. aureus* were susceptible to amoxicillin, chloramphenicol, kanamycin, gentamicin, cephalexin, and tetracycline. Most bacterial isolates were resistant to the six commonly used antibiotics. The concentration of intermediates should be increased to be effective again while those which were resistant should be replaced.

Keywords antibiotic susceptibility patterns, pathogenic bacteria

Introduction

Antibiotics have been available in the environment much before humans started employing them in clinical settings and many microorganisms have naturally been exposed to these bioactive compounds during the evolution process [1]. Antibiotics have also evolved to be global regulators within microbial communities, contributing to quorum sensing and microbial communication in the natural milieu [2, 3]. At very low concentrations, antibiotics can act as signaling molecules and triggers the transcription responses that are important for environmental survival [4, 5]. A competitive role is only achieved once the antibiotic concentration is high enough to inhibit the growth of surrounding microorganisms [6]. Transient high antibiotic concentrations in nature made it necessary for antibiotic-producing organisms to harbor resistance genes needed for self-protection and stimulated the evolution of resistant genes in neighboring bacteria [7].

Antimicrobial resistance was recognized soon after the discovery of penicillin [8] and later the introduction of every new drug was followed. However, antibiotic resistance was not regarded as a serious problem for a long time in large parts because it was possible to find an effective therapy due to the availability of many different classes of antibiotic [9]. The resistance did not rule out the therapy since newer drug derivatives with higher potency were being developed [10].

The antibiotics used in the treatment of dairy animals have got their way into the milk thus, leading to the emergence of antimicrobial-resistant bacterial strains. The transfer of bacterial resistance to antibiotics from animals to humans has become a global threat [12]. The antibiotic residues in milk, which can initiate severe reactions in people allergic to antibiotics and moreover, at a low level can cause sensitization of normal individuals and the development of antibiotic resistant strains of bacteria [13]. A growing body of evidence supports the concept that the amount of antibiotics used in animals has an impact on the levels of resistant bacteria in humans [14]. Antibiotic resistance has emerged as one of the most severe contemporary health care problems in community and hospital settings and poses a serious threat to our ability to treat bacterial infections. If this trend continues, we might soon approach a post-antibiotic era, in which bacterial infections that could be cured easily for more than sixty years are once again untreatable [15].

Methodology

Antimicrobial Response Tests (AST): Bacterial isolates obtained were examined for antibiotic resistance using the standard Kirby-Bauer disk diffusion method. The antibiotics tested were; tetracycline, chloramphenicol, cephalexin, gentamicin, kanamycin, and amoxicillin. Mueller – Hinton medium plates were swabbed (cotton swabs of 0.1ml as per manufacturer's recommendation) with the inoculums and the six commercially prepared antimicrobial agent disks were placed on each of the inoculated plates. The plates were incubated at 37 °C for 24hours. The diameters of clear zones of growth inhibition around the antimicrobial agent disks, including the 6 mm disk diameter were measured by using precision calipers (Clinical and Laboratory Standards Institute (CLSI) and were compared to the standard reference organisms. The break-points used to categorize the isolates as resistant to each antimicrobial agent were those recommended by CLSI (2016).

Data analysis

Means and percentages were used to describe the occurrence of antimicrobial resistance. The difference in response to antibiotics and levels of antibiotics between and within the groups in the study was assessed using analysis of variance (ANOVA). Statistical significance was set at $p<0.05$ using a computer package, SPSS software version 20.0.

Results and Discussion

Susceptibility patterns for the six isolated bacterial pathogens: What is an antibiotic? An antimicrobial substance naturally produced by bacteria or fungi that can kill or inhibit the growth of other microorganisms. People make use of many types of antibiotics as medicines to prevent and treat infections caused by pathogenic bacteria, fungi, and certain parasites. The majority of antibiotics are primarily used against bacteria [16]. The means which represent the diameter of the zone of inhibition for each bacterial species is the average of the number of isolates since the study did not identify different bacterial serotypes. The tables below show the susceptibility patterns of the six isolated bacteria (*S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *C. freundii*, *B. subtilis*) against 6 antibiotics (amoxicillin, chloramphenicol, kanamycin, gentamicin, cephalexin, and tetracycline). The concentration of antibiotic is given in µg. Means represent the diameter of the zone of inhibition from triplicates, n = Total Number of tests. The isolated organism was compared to standard reference organism.

Table 1. Sensitivity patterns of *Staphylococcus aureus*

Staphylococcus aureus	**AMX 30 µg**	**K 30µg**	**GEN 10µg**	**CN 5 µg**	**C 50µg**	**TE 30µg**
Mean	18.27	21.68	22.32	22.95	29.50	23.36
Number of isolates	66	66	66	66	66	66
Resistant	≤13	≤13	≤12	≤15	≤12	≤14
Intermidiate	14-17	14-17	13-14	16-20	13-17	15-18
Susceptible	≥18	≥18	≥15	≥21	≥18	≥19

Standard reference organism (ATCC25923)

Figure 1. *Staphylococcus aureus* sensitivity patterns

Staphylococcus aureus is still susceptible to tetracycline, chloramphenicol, cephalexin, gentamicin, kanamycin, and amoxicillin

Table 2. Sensitivity patterns of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa	**AMX 30 µg**	**K 30µg**	**GEN 10µg**	**CN 5 µg**	**C 50µg**	**TE 30µg**
Mean	6.00	10.93	21.30	6.00	21.63	6.80
Number of isolates	30	30	30	30	30	30
Resistant	≤13	≤13	≤12	≤15	≤12	≤11
Intermidiate	14-17	14-17	13-14	16-20	13-17	12-14
Susceptible	≥18	≥18	≥15	≥21	≥18	≥15

Standard reference organism (ATCC27853)

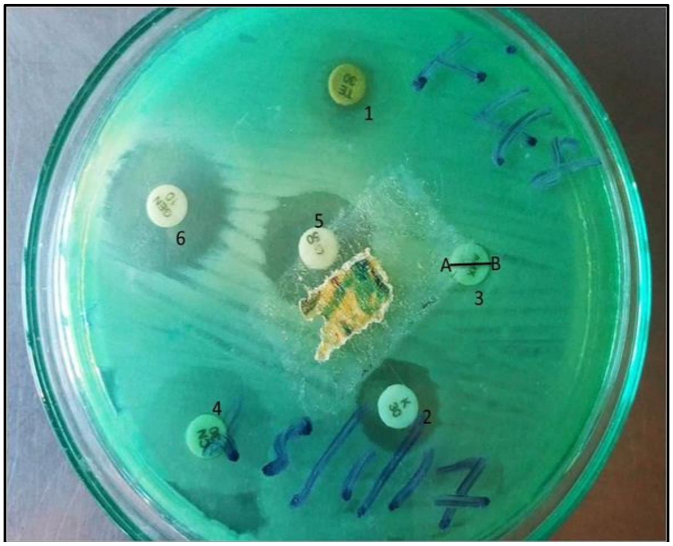

Figure 2. ***Pseudomonas aeruginosa*** **sensitivity patterns**

Pseudomonas aeruginosa is resistant to tetracycline, cephalexin, kanamycin, and amoxicillin but susceptible to gentamicin and chloramphenicol

Table 3. Sensitivity patterns of ***Escherichia coli***

Escherichia coli	**AMX 30 µg**	**K 30µg**	**GEN 10µg**	**CN 5 µg**	**C 50µg**	**TE 30µg**
Mean	**6.00**	17.84	19.90	**16.59**	28.63	20.76
Number of isolates	51	51	51	51	51	51
Resistant	≤13	≤13	≤12	≤15	≤12	≤11
Intermidiate	14-17	14-17	13-14	16-20	13-17	12-14
Susceptible	≥18	≥18	≥15	≥21	≥18	≥15

Standard reference organism (ATCC35218)

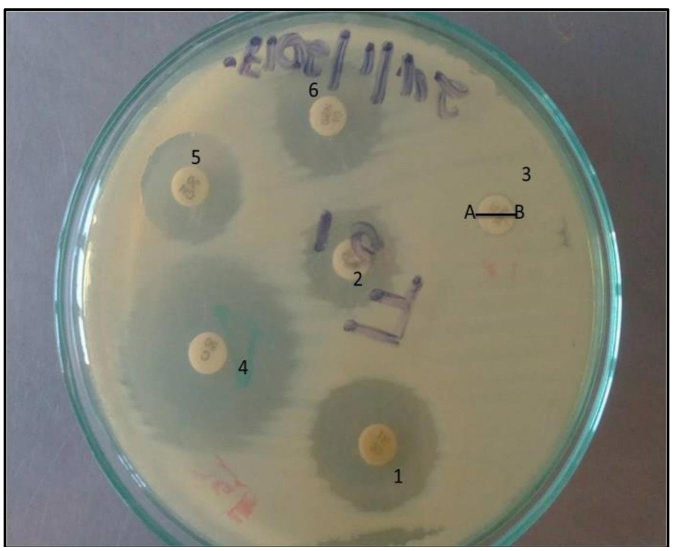

Figure 3. ***Escherichia coli*** **sensitivity patterns**

Escherichia coli is resistant to amoxicillin, intermediate to cephalexin and kanamycin, but susceptible to gentamicin, chloramphenicol, and tetracycline. The concentration of cephalexin and kanamycin can be increased in order to use it against *E coli*.

Table 4. Sensitivity patterns of *Klebsiella pnemoniae*

Klebsiella pnemoniae	**AMX 30 µg**	**K 30µg**	**GEN 10µg**	**CN 5 µg**	**C 50µg**	**TE 30µg**
Mean	22.71	22.81	23.67	21.71	27.71	23.43
Number of isolates	21	21	21	21	21	21
Resistant	≤13	≤13	≤12	≤15	≤12	≤11
Intermidiate	14-17	14-17	13-14	16-20	13-17	12-14
Susceptible	≥18	≥18	≥15	≥21	≥18	≥15

Standard reference organism (ATCC700603)

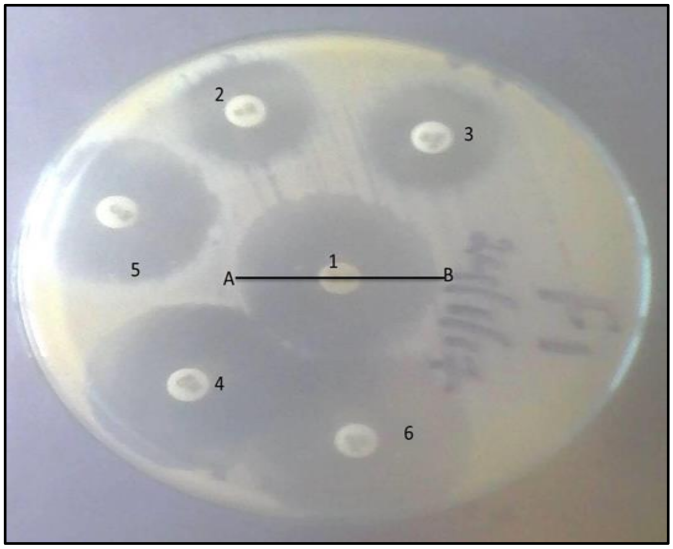

Figure 4. *Klebsiella pnemoniae* sensitivity patterns

Klebsiella pnemoniae is susceptible to gentamicin, chloramphenicol, tetracycline, cephalexin, amoxicillin, and kanamycin.

Table 5. Sensitivity patterns of *Citrobacter freundii*

C. freundii	**AMX 30 µg**	**K 30µg**	**GEN 10µg**	**CN 5 µg**	**C 50µg**	**TE 30µg**
Mean	6.00	19.37	20.79	6.00	23.12	19.46
Number of isolates	24	24	24	24	24	24
Resistant	≤13	≤13	≤12	≤15	≤12	≤11
Intermidiate	14-17	14-17	13-14	16-20	13-17	12-14
Susceptible	≥18	≥18	≥15	≥21	≥18	≥15

Standard reference organism (ATCC8090)

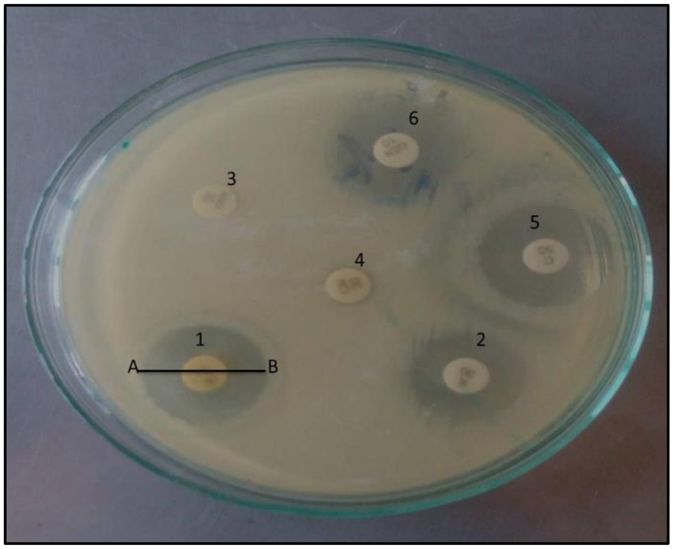

Figure 5. ***Sensitivity patterns of Citrobacter freundii***

Citrobacter freundii is resistant to amoxicillin and cephalexin but susceptible to gentamicin, chloramphenicol, tetracycline, and kanamycin.

Table 6. Sensitivity patterns of *Bacillus subtilis*

Bacillus subtilis	**AMX 30 µg**	**K 30µg**	**GEN 10µg**	**CN 5 µg**	**C 50µg**	**TE 30µg**
Mean	**6.00**	**17.11**	19.85	**12.48**	25.52	18.56
Number of isolates	27	27	27	27	27	27
Resistant	≤13	≤13	≤12	≤15	≤12	≤11
Intermidiate	14-17	14-17	13-14	16-20	13-17	12-14
Susceptible	≥18	≥18	≥15	≥21	≥18	≥15

Standard reference organism (ATCC23857)

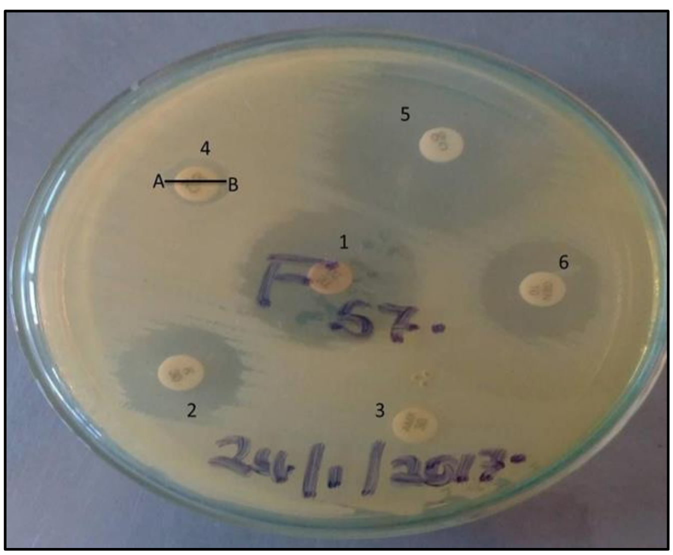

Figure 6. ***Bacillus subtilis*** **of Sensitivity patterns**

Bacillus subtilis is resistant to amoxicillin and cephalexin, intermediate to kanamycin, but susceptible to gentamicin, chloramphenicol, and tetracycline. The concentration of kanamycin can be increased in order to use it against B. subtilis.

Table 7. The table showing percentages of bacterial susceptibility patterns to antibiotics

Antibiotics	Percentage (%) of bacterial resistance to antibiotics	Percentage (%) of bacterial intermediate to antibiotics	Percentage (%) of bacterial susceptibility to antibiotics
Amoxicillin	63%	0%	37%
Cephalexin	41%	22%	37%
Kanamycin	19%	33%	48%
Tetracycline	19%	0%	81%
Chloramphenicol	0%	0%	100%
Gentamicin	0%	0%	100%

Percentages of bacteria resistant to antibiotics were amoxicillin (63%), kanamycin (19%), cephalexin (41%) and tetracycline (19%). Kanamycin (33%) and cephalexin (22%) were intermediate. Susceptible ones were amoxicillin (37%), gentamicin (100%), kanamycin (48%), cephalexin (37%), chloramphenicol (100%) and tetracycline (81%). In general, 62% of the bacteria were resistant, 33% were intermediate while 5% were susceptible. The percentage of bacteria resistant to antibiotics was extremely high (62%). This trend explains the reason why mastitis infections are rampant within Ndivisi ward reducing the efficacy of the available and commonly used antibiotics. It can be observed from the table above that chloramphenicol and gentamicin are the antibiotics of choice that can be used since they have an efficacy of 100%.

Conclusion

Bacterial antibiotic resistance makes the treatment of infectious diseases difficult as it reduces the effectiveness of the available drugs. Antimicrobial resistance occurs mainly as a consequence of selection pressure placed on the susceptible microbes by the use of antimicrobial agents, environmental contamination with excreted antimicrobials or their metabolites, residue concentrations of antimicrobials in edible tissues, and direct zoonotic transmission.

Awareness

Bacterial resistance to antibiotics pose serious problems that must be addressed as a matter of agency.

Acknowledgements

I am grateful to my supervisors; Dr. Mario Kollenberg and Prof. Donald Siamba for their sincere encouragement and guidance. I also thank Mr. Peter Nyongesa who assisted me through the entire research.

Abbreviations

Tetracycline (TE)
Cephalexin (CN)
Kanamycin (K)
Chloramphenicol (C)
Amoxicillin (AMX)
Gentamycin (GEN)

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