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Antibiotics Resistance and Plasmid Curing of Listeria Monocytogens Isolates from Milk and Milk Products

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Abstract: This study investigated the prevalence and antibiotic resistance profiles of Listeria monocytogenes in milk and milk products, given its role in the foodborne illness listeriosis. Twelve milk samples were collected and processed using standard microbiological techniques. Listeria isolates were identified through biochemical characterization and reference to Bergey's Manual of Systematic Microbiology. Antibiotic susceptibility was assessed adopting the Kirby-Bauer method, and Multiple Antibiotic Resistance (MAR) indices were calculated. Plasmid profiling was conducted via PCR, followed by a curing process and post-curing sensitivity testing on isolates carrying resistance plasmids. Listeria was isolated from four of the twelve samples, specifically from nunu, yogurt, cheese, and cow milk. The isolates exhibited varying levels of resistance to the tested antibiotics. Two isolates displayed MAR indices of 0.41, indicating multidrug resistance. Plasmid profiling detected a plasmid in one isolate. Following the curing process, the isolate harboring the plasmid became susceptible to erythromycin, an antibiotic to which it was previously resistant, suggesting plasmid-mediated resistance. Conversely, an isolate without a detectable plasmid remained resistant after curing, implying a non-plasmid-mediated resistance mechanism. Notably, both isolates (YS and CA) remained resistant to cephalosporin after curing. All isolates demonstrated full susceptibility to gentamycin. This study confirmed the presence of Listeria species in some of the milk products tested.

Keywords: Milk, Milk products, antibiotic resistance, plasmids, plasmid curing.

Introduction

The genus Listeria encompasses ten closely related species of rod-shaped, Gram-positive bacteria that are facultative anales, non-spore-forming, and motile (Zhang et al., 2007). While most Listeria species are harmless, two notable exceptions - L. monocytogenes and L. ivanovii - are pathogenic and can cause listeriosis (Liu, 2006). L. monocytogenes, in particular, is a formidable foodborne pathogen that can invade host cells, leading to severe infections with high mortality rates, especially among vulnerable populations such as pregnant women, newborns, the elderly, and immunocompromised individuals. This bacterium has been isolated from a wide range of sources, including environmental samples and various foods (Liu, 2008). The widespread presence of L. monocytogenes in food presents a substantial risk. Although L. monocytogenes has historically exhibited relatively stable antibiotic susceptibility, recent reports, including those from Nigeria (Enurah et al., 2013), document the emergence of antibiotic-resistant strains isolated from both food and environmental sources. Plasmid presence has been linked to this antibiotic resistance in L. monocytogenes (Enurah et al., 2013).

Listeria monocytogenes poses a significant challenge to dairy production due to its ability to form biofilms that can evade standard cleaning and disinfection protocols. The risk of contamination is heightened by the potential for carrier animals to shed the bacteria, thereby increasing the likelihood of contamination in raw milk and other dairy products (Hunt et al., 2012). Moreover, the rising antibiotic resistance of L. monocytogenes in dairy products raises critical public health concerns, as it may severely impede the management of future outbreaks and disease transmission (Beyza and Kefyalew, 2019).

The escalating antibiotic resistance crisis has far-reaching implications, compromising the efficacy of antibiotic treatments and hindering the development of novel antimicrobial agents (Li and Nikaido, 2009). This has led to the rapid dissemination of resistant bacteria and resistance genes across human, animal, and environmental sectors worldwide (Rousham et al., 2018). Despite the pressing need for action, research efforts to address this challenge remain inadequate.

Studies focusing on Listeria monocytogenes have revealed troubling trends, including the identification of over 14 serotypes and a Multiple Antibiotic Resistance (MAR) index exceeding 0.2. The reported average plasmid profile of 54% is particularly worrisome, suggesting a substantial risk of widespread antibiotic resistance dissemination (Schmitz-Esser, 2021).

Plasmids are autonomously replicating, circular DNA molecules separate from a host's chromosomal DNA, and are present in characteristic copy numbers (Rajashree et al., 2018). These extrachromosomal elements frequently carry genes responsible for antibiotic resistance and the breakdown of antimicrobial compounds. Critically, plasmids can mediate the horizontal transfer of resistance genes between bacteria through processes like transformation, conjugation, and mobilization (Opal et al., 2000). This horizontal gene transfer plays an important role in the dissemination of antibiotic resistance and can, in some instances, increase the virulence of recipient bacterial cells.

2.0. Materials and Methods

2.1. Collection of Samples.

Seven milk and milk product samples were purchased from shops in Owo, Ondo State, and transported to the Microbiology Laboratory, Department of Biological Sciences of Achievers University Owo for analysis. The samples included pasteurized milk, goat milk, cow milk, sheep milk, cheese, Nunu, and yogurt. All samples were refrigerated until testing.

2.2. Isolation from samples

Serial dilutions (10^6) were performed for all samples. One milliliter of each diluted sample was then pour-plated using pre-prepared, sterilized Mueller-Hinton agar. Plates were incubated aerobically and anaerobically (in an anaerobic jar) for 24-48 hours. Pure cultures were obtained from the resulting isolates and incubated for an additional 24 hours. These pure cultures were then stored on nutrient agar slants until further use. Fresh broth cultures were also prepared from each slant to facilitate subsequent analyses.

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2.3. Characterization and identification.

Characterization and identification of isolates were carried out using standard colonial, morphological and biochemical procedures as described in literature.

2.3.1. Gram staining.

This was carried out adopting the procedure described by Hitchins et al., (2022).

2.3.2. Haemolysis /Pathogenicity Test.

The method described by Jinneman et al., (2022) was adopted in this test; A loopful of a culture of L. monocytogenes used to streak blood agar, then incubated at 35oC for 48 hr. The plates were read after the period of incubation and the result recorded.

2.3.3. Sugar utilization

This test was carried out using sugar such as lactose, maltose, fructose, galactose, glucose, sucrose, ribose and arabinose. Peptone broth containing 0.5percent fermentable sugar was prepared by pouring the sugar into the broth. A 0.03g aliquot of bromocresol indicator was added and mixed together, Durham tubes are inserted into each test to collect gas. The test tubes are plugged in properly with cotton wool and were autoclaved at 121oC for 15min after which the test tubes are allowed to cool and the isolates are aseptically inoculated onto test tubes. A positive result was evidenced by colour change indicating acid production and gas in the Durha tubes (the test tubes are incubated at 37oC for 24hrs) (Procop et al., 2017).

2.3.4. Oxidase test

A loopful of colony was picked from the petri dish after incubation and smeared on the filter paper with oxidase reagent (Kovacs Oxidase Reagent). The change of colour from white into deep purple after 10~15 seconds indicated a positive reaction (Chen et al., 2022).

2.3.5. Catalase test

The catalase test, using the slide method, began with the transfer of a minute amount of L. monocytogenes colony onto a clean, dry glass slide using a wire loop. A single drop of 3% hydrogen peroxide was then added to the slide. The resulting reaction was observed for the production of oxygen bubbles. The evolution of bubbles indicated a positive catalase reaction (Aryal, 2022)

2.3.6. The Voges-Proskauer (VP) test

The Voges-Proskauer (VP) test procedure begins with allowing the medium to cool room temperature before inoculation. A light inoculation of L. monocytogenes, taken from a pure 18–24-hour culture, is then introduced to the medium. The inoculated plate is incubated under aerobic condition at 37°C for 24 hours. After this initial incubation period, a 2 ml aliquot of the broth was transferred to a test tube, while the remaining broth is re-incubated for another 24 hours. To the 2 ml aliquot, 6 drops of 5% alpha-naphthol are added and mixed thoroughly to aerate the solution. Subsequently, 2 drops of 40% potassium hydroxide are added, followed by another thorough mixing to aerate. Within 30 minutes, the reaction is observed for the development of a pink-red color at the surface. Throughout this 30-minute period, the tube is shaken vigorously.

2.3.7. Indole Test.

The Indole test for L. monocytogenes can be performed using either a broth method or a spot test

method.

Broth Method:

Aseptically, 4 ml of tryptophan broth in a sterile test tube is inoculated with growth from an 18-24h culture of L. monocytogenes. The inoculated tube is then incubated at 37°C for 24-28 hours. Following incubation, 0.5 ml of Kovac's reagent is added to the broth culture. The presence or absence of a ring is then observed. A ring indicates a positive result for L. monocytogenes.

2.3.8. Motility Test.

The motility test for L. monocytogenes involves a stab inoculation 18-24h culture grown on agar medium. Using a needle, the culture is stabbed into the agar and then incubated at 20°C for 24-48 hours. A positive result is indicated by the migration of the bacteria away from the stab line. Conversely, a negative result would show growth concentrated along the stab line with a clearer surrounding medium.

2.3.9. Antibiotic sensitivity Test;

Isolates were subjected to antibiotic susceptibility testing using the standadized Kirby Bauer method

evaluated by the methods of Clinical Laboratory standards Institute (CLSI) (Hudzicki, 2009).

2.3.9.1. Standardization of Inoculum

Bacterial cultures were grown on nutrient agar plates at 37°C for 24 hours. A 0.1 ml number of bacterial cells was then suspended in sterile normal saline. A 0.5 McFarland standard turbidity was achieved using a barium sulfate solution prepared by combining 0.6 ml of 1% barium chloride with 99.4 ml of sulfuric acid (CLSI, 2016). This bacterial suspension was spread onto Mueller-Hinton agar plates using the spread plate procedure. Antibiotic discs were immediately placed on the agar surface using sterile forceps. The plates were incubated aerobically at 37°C for 16 hours. Thereafter, zones of inhibition were measured, and the results were recorded and interpreted according to the ClSI standards (CLSI, 2012).

2.3.10. Multiple Antibiotic resistance index (MARI)

The resistance index will be obtained by dividing the number of antibiotics that the isolate is resistant to by the number of antibiotics tested (Osundiya et al., 2013).

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2.3.11. Methods for extraction and purification of bacterial DNA

An overnight cultures of various Listeria monocytogenes strains in Brain Heart Infusion (BHI) broth, Buffered Listeria Enrichment Broth (BLEB), or enrichment samples were centrifuged at 12,000g for 3 minutes. The resulting cell pellets were washed, resuspended in sterile distilled water or Tris-EDTA (TE) buffer, and subjected to heat treatment in a boiling water bath or heat block for 10 minutes. After cooling on ice for at least 5 minutes, the samples were centrifuged again at 12,000g for 10 minutes. The supernatants containing the crude bacterial DNA templates were then collected. Subsequently, 1-5 µL of these supernatants were used directly for polymerase chain reaction (PCR) amplification (Jung et al., 2003; Jamali et al., 2013).

2.13. Plasmid curing procedure

Plasmid curing experiments were performed using a modified method of Deshpande et al. (2001). Cultures grown overnight (0.2 ml) were introduced into 5 ml of nutrient broth supplemented with 10% sodium dodecyl sulfate (SDS). The resulting mixtures were incubated at 37°C for 24 hours. After incubation, the cultures were thoroughly mixed and subsequently sub-cultured onto Mueller-Hinton agar (MHA) plates. These plates were then incubated at 37°C for an additional 24 hours.

3.0 Results

3.1. Heterotrophic Plate Count of Bacterial Isolates

Table 1 shows the heterotrophic plate count (HPC) of bacteria from different samples of milk and milk product. The HPC in all the samples ranged between 1.0 × 107 and 10 × 107 CFU/ml. Nunu sample had the highest colony count of 4.0 × 107 CFU/ml, followed by Cow milk (2.5 × 107 CFU/ml) and Goat milk (1.5× 107 CFU/ml) samples respectively, while sheep milk, pasteurized milk and yoghurt samples had the lowest bacteria count of 1.0 × 107 CFU/ml.

3.2. Identification of isolates

Listeria Spp were isolated from three (3) samples viz-a-viz nunu, yoghurt and cow milk. The anaerobically incubated, nunu, yoghurt and cow milk yielded more isolates than the aerobically incubated samples (Table 2). The isolates were identified as rod shaped, Gram positive, Vogues Proskeur positive, Methyl red positive, catalase positive, motile, exhibiting +β heamolysis and were able to ferment glucose, lactose, maltose and sucrose (Table 2).

3.3. Antimicrobial Susceptibility Test

Table 3 revealed the susceptibility and resistance profile of Listeria species, isolated from milk and milk products. The organisms showed different degrees of susceptibility to different antibiotics that were used in this work. Listeria sp., isolated from Nunu was resistant to three antibiotics, namely: cefuroxime, cefotaxime and cefexime, while susceptibility was shown to impenem/cilastatin, amoxicillin / clavulanic acid, ceftriaxone sulbactarm, levofloxacin, ciprofloxacin, erythromycin, gentamycin and azithromycin. Also, the Listeria sp., isolated from Yoghurt was resistant to five antibiotics, which include: cefotaxime, ceftriaxone sulbactarm, cefexime, amoxicillin / clavulanic acid and erythromycin while Listeria sp. that was isolated from Cow milk was resistant to impenem/cilastatin, amoxicillin / clavulanic acid and erythromycin. The Listeria sp. that was isolated anaerobically from yoghurt was resistant to impenem, cefotaxime, cefexime and amoxicillin / clavulanic acid.

3.4. Multiple antibiotic resistance status of isolates

The Multiple antibiotic resistant (MAR) index of bacterial isolates (Listeria sp.) ranged between 0.25 to 0.41.

3.5. Plasmid Profiling of Resistant Isolates

Figure 1 shows the electrophoretic gel result of plasmid profiling of resistant isolates. Isolate YS and CS demonstrated high resistance to some antibiotics investigated in this study, with MAR index of 0.4.1. Isolates YS as represented as Y in Figure 1 had high molecular weight plasmid with band size greater than 1500bp while plasmid was absent in isolate CA

3.6. Curing of plasmid of resistant isolates;

Figure 2 reveals the absence of plasmid after curing on agarose gel. The antibiotic sensitivity pattern before and after plasmid curing of multidrug resistant isolate was revealed in Table 4. The isolates displayed resistance to antibiotics even after curing except for isolate with plasmid that was susceptible to erythromycin after curing.

4.0. Discussion

Listeria monocytogen is a dangerous pathogenic bacterium to man and animals. The organism can grow at refrigeration temperature and thus can be a potential microbiological risk for various refrigerated foods such as milk and milk products. Milk is a staple food consumed globally, valued for its rich nutritional content. However, this nutritional richness also makes milk an excellent medium for microbial growth, including the potential proliferation of pathogenic organisms such as Listeria monocytogenes (L. monocytogenes) (Balthazar et al., 2017).

Raw milk's rich nutritional composition, near-neutral pH, and elevated water activity make it a favorable environment for the proliferation of various microorganisms. The extent of this microbial growth is largely influenced by temperature, the presence of competing microorganisms, and the resulting metabolic byproducts.

One of the safety measures for quality assurance of milk and milk products is by checking for the presence of pathogenic microorganisms. L. monocytogenes is a

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human pathogen considered to be a major microbiological hazard associated with the consumption of raw cow milk (Verraes et al., 2015; Claeys et al., 2013). Several authors have reported the prevalence of Listeria spp. and Listeria monocytogenes in milk and dairy products (Buchanan et al., 2017; Chen et al., 2015; Nayak et al., 2015). The prevalence of L. monocytogenes in milk varies considerably among several research reports and has been attributed to factors like environmental conditions, farm size, farm management practices, sampling and detection methods used among others (Claeys et al., 2013). In addition, standard plate count (SPC) or heterotrophic plate count (HPC) of bacteria has also been proven to be effective in the evaluation of food safety.

In this study, the heterotrophic plate count revealed the presence of heterotrophic bacteria in all samples examined. The colony count was above the standard plate count (2× 105 cfu/ml). A study by Savadogo et al. (2004) revealed high colony count of bacteria in all samples ranging from 1.02 × 102 to 9.89 × 102 cfu/ml.

Studies on L. monocytogenes prevalence reveal varying levels of contamination across different regions. In Africa, reported incidence rates range from 0% to 22.0%, primarily in raw cow milk samples, though analyses have also included pasteurized cow milk, as well as milk from camels, sheep, and goats. A New Zealand study detected L. monocytogenes in 2 out of 297 milk samples tested (0.7%) (Amagliani et al., 2012). Middle Eastern studies show a wider range of incidence, from 0% to 50%, again predominantly in raw cow milk, but also encompassing pasteurized cow milk and milk from camels, sheep, buffalo, and goats. It is worth noting that one study, based on a small sample size of 18, reported a high incidence of 50% (9 positive samples) (Buchanan, 2017). Similarly, in Asia, reported L. monocytogenes incidence varies between 0% and 25%. A study analyzing only 5 raw milk samples showed a 25% incidence, highlighting the potential for high contamination even in small samples (Muthulakshmi, 2018). The presence of L. monocytogenes in traditional foods may be attributed to suboptimal manufacturing practices and production processes. Key risk factors contributing to contamination include the use of raw materials, distribution of unpasteurized end products, and inadequate fermentation and storage conditions.

In this study, 12 randomly collected samples were cultured, revealing a 33.33% (4/12) prevalence of Listeria sp. This rate aligns with findings from other researchers. Molla et al. (2004) reported a 32.6% Listeria prevalence, Firehiwot (2007) found 27.4%, and Seyoum et al. (2015) observed 28.4% across various food types. Conversely, our results are higher than those reported by Muhammed et al. (2013), who found a 6.5% prevalence in milk and milk products. These variations in Listeria prevalence across studies may be due to differences in sample types, food composition, or the hygiene standards of production and processing facilities. Furthermore, the growth of the pathogen both in aerobic and anaerobic conditions confirms that it is a facultative anaerobe.

The antibiotic susceptibility test conducted in this study showed that most Listeria species isolates were susceptible to the antibiotics used, with the notable exception of cefexime, to which all isolates were resistant, and amoxicillin/clavulanic acid, to which 75% of isolates were resistant. The emergence of antibiotic resistance is primarily attributed to the overuse and misuse of antibiotics in humans and animals, as well as inadequate infection-prevention measures (Montalti et al., 2022).

Fortunately, the majority of Listeria spp. isolated in this study were sensitive to gentamicin, which is commonly used in combination with ampicillin or oxacillin to treat listeriosis. Combination therapy involving gentamicin, tetracycline, erythromycin, and trimethoprim-sulfamethoxazole is also considered a frontline treatment for listeriosis in animals (Luque-Sastre et al., 2018). However, the isolates exhibited 50% resistance to erythromycin, which had previously been effective against listeriosis.

The multiple antibiotic resistance (MAR) indices of Listeria species in this study indicate moderate resistance, although the isolates showed greater susceptibility to the antibiotics used. The rising threat of antimicrobial resistance (AMR) poses a significant public health crisis in the 21st century, compromising the effectiveness of treatments for various infections and underscoring the need for judicious antibiotic use and the development of novel antimicrobial agents.

This study detected a plasmid in one isolate but not in the other. The presence of a plasmid can contribute to antibiotic resistance and its potential transmission between bacteria, as noted by Alanazi et al. (2018). Conversely, the absence of a plasmid suggests that any observed resistance may be chromosomally encoded or intrinsic to the organism.

Plasmid curing experiments, followed by sensitivity testing, demonstrated that erythromycin resistance in one isolate was plasmid-mediated, as susceptibility to the antibiotic was restored after plasmid elimination. Both isolates, however, remained resistant to cefexime, cefuroxime, and cefotaxime even after curing. These antibiotics belong to the cephalosporin group, which has long been recognized as ineffective against Listeriosis (Hof, 2003). The intrinsic resistance of L. monocytogenes to cephalosporins is generally attributed to the absence of penicillin-binding proteins (PBPs) that can effectively bind and be inhibited by these beta-lactam antibiotics (Krawczyk-Balska and Markiewicz, 2016; Poros-Gluchowska and Markiewicz, 2003).

In the isolate where resistance was maintained post-curing, the resistance mechanism does not appear to be plasmid mediated. This suggests that the observed resistance in this isolate is likely chromosomally encoded (Letchumanan et al., 2015).

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Conclusion

Milk is a major source of animal protein in diets worldwide, making it economically and nutritionally significant. The presence of substantial levels of pathogenic and antibiotic-resistant bacteria in milk and milk products poses a risk to human health. This study detected Listeria species, which were generally susceptible to most tested antibiotics, with the exception of cephalosporins, against which 100% resistance was observed. The findings also suggest that plasmids may play a role in mediating antibiotic resistance in these bacteria.

Appendices

Table 1: Heterotrophic Plate Count of Bacterial from Different Milk Samples

Samples/Sources	Colony forming unit(CFU/ml) = number of colonies × Total dilution factor / Volume of Culture plated
Sheep Milk	1.0×10^7
Nunu	4.0×10^7
Cow milk	2.5×10^7
Goat milk	1.5×10^7
Pasteurized milk	1.0×10^7
Yoghurt	1.0×10^7

Table 2. Identification of Listeria Isolates

Sample Code	Gram reaction	Cellular Morphology	Voges Proskauer	Methy Red (MR)	Catalase	Oxidase	Motility	Indole	Haemolysis	Sugar Fermentatio						H_2S	Gas	Probable organisma
											Lac	Glu	Mal	Suc	Rib			
NS	+ve	R	+	+	+	-	+	-	+β	+	+	+	+	-	-	-	-	*Listeria* sp
YS	+ve	R	+	+	+	-	+	-	+β	+	+	+	+	-	-	-	-	*Listeria* sp
NA	+ve	R	+	+	+	-	+	-	+β	+	+	+	+	-	-	-	-	*Listeria* sp
CA	+ve	R	+	+	+	-	+	-	+β	+	+	+	+	-	-	-	-	*Listeria* sp
YA	+ve	R	+	+	+	-	+	-	+β	+	+	+	+		-	-	-	*Listeria* sp

Legend.

NS; Nunu under aerobic incubation

YS; Yoghurt under aerobic incubation

NA; Nunu under anaerobic incubation

CA; Cow milk under anaerobic incubation

YA; Yoghurt under anaerobic incubation

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Table 3. Antibiotic resistance status of isolates

Isolates	IMP	CXM	GN	CTX	CRO	ZEM	LBC	CIP	AZM	AUG	ERY	OFX	MAR Index
NS	15I	11R	22S	12R	16S	-R	30S	30S	30S	30S	30S	30S	0.25
YS	20S	18S	18S	08R	16R	-R	30S	30S	22S	-R	13R	25S	0.41
NA													
CA	08R	17S	20S	09R	18S	-R	30S	30S	20S	-R	-R	30S	0.41
YA	12R	18S	22S	10R	16S	-R	30S	30S	21S	-R	16S	30S	0.3

IMP- Impenem/cilastatin, CXM- Cefuroxime, AUG- Amoxicillin / clavulanic acid, CTX- Cefotaxime, CRO- Ceftriaxone Sulbactarm, ZEM- cefexime, LBE- Levofloxacin, CIP- Ciprofloxacin, OFX- Ofloxacin, ERY- Erythromycin, GN- Gentamycin, AZN- Azithromycin.

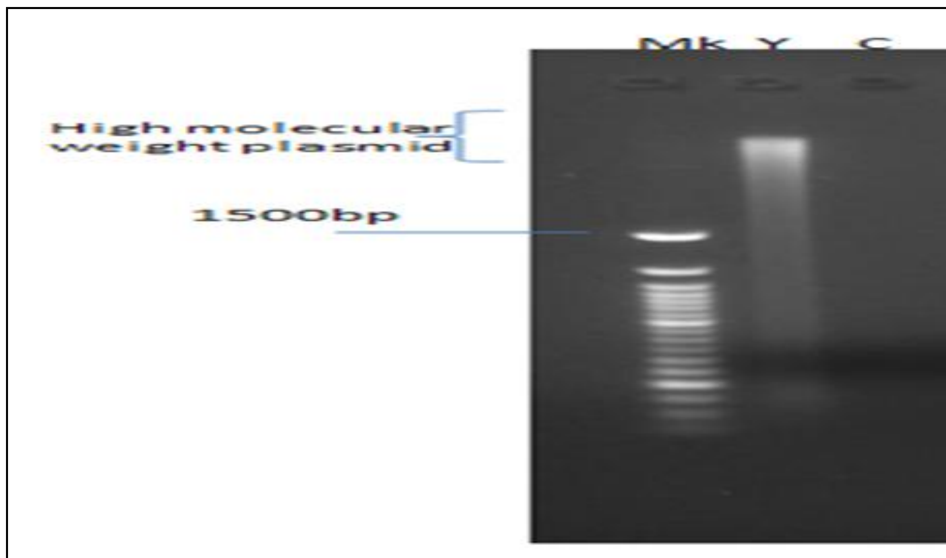

Figure 1: Electrophoretic gel result of plasmid profiling of resistant isolates

Key: MK- Ladder, Y- isolate YS, C- Isolate CS

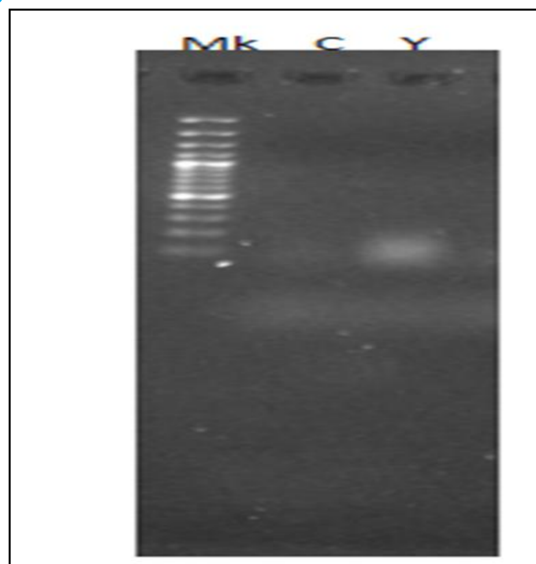

Figure 2: Agarose gel showing the absence of Plasmid after curing.

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Table 4: The Antibiotic Sensitivity Pattern Before and After Curing of Plasmids of the Multidrug Resistant Isolates.

Isolate code		IMP	CXM	GN	CTX	CRO	ZEM	LBC	CIP	AZM	AUG	ERY	OFX
YS	Bf	S	R	S	R	R	R	S	S	S	R	R	S
	Aft	S	R	S	R	R	R	S	S	S	R	**S**	S
CA	Bf	S	R	S	R	S	R	S	S	S	R	R	S
	Aft	S	R	S	R	S	R	S	S	S	R	R	S

Legend
R- Resistant
S.- Susceptible.

References

1. Amagliani, G., Petruzzelli, A., Omiccioli, E., Tonucci, F., Magnani, M., & Brandi, G. (2012). Microbiological surveillance of a bovine raw milk farm through multiplex real-time PCR. *Foodborne Pathogens and Disease*, *9*(5), 406-411.
2. Aryal, J., Gautam, B., & Sapkota, N. (2012). Drinking water quality assessment.
3. Balthazar, C. F., Pimentel, T. C., Ferrão, L. L., Almada, C. N., Santillo, A., Albenzio, M., ... & Cruz, A. G. (2017). Sheep milk: Physicochemical characteristics and relevance for functional food development. *Comprehensive reviews in food science and food safety*, *16*(2), 247-262.
4. Ulusoy, B. H., & Chirkena, K. (2019). Two perspectives of Listeria monocytogenes hazards in dairy products: the prevalence and the antibiotic resistance. *Food Quality and Safety*, *3*(4), 233-241.
5. Buchanan, R. L., Gorris, L. G., Hayman, M. M., Jackson, T. C., & Whiting, R. C. (2017). A review of Listeria monocytogenes: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food control*, *75*, 1-13.
6. Caruso, M., Fraccalvieri, R., Pasquali, F., Santagada, G., Latorre, L. M., Difato, L. M., ... & Parisi, A. (2020). Antimicrobial susceptibility and multilocus sequence typing of Listeria monocytogenes isolated over 11 years from food, humans, and the environment in Italy. *Foodborne pathogens and disease*, *17*(4), 284-294.
7. Chen, M., Wu, Q., Zhang, J., Wu, S., & Guo, W. (2015). Prevalence, enumeration, and pheno-and genotypic characteristics of Listeria monocytogenes isolated from raw foods in South China. *Frontiers in microbiology*, *6*, 1026.
8. Claeys, W. L., Cardoen, S., Daube, G., De Block, J., Dewettinck, K., Dierick, K., ... & Herman, L. (2013). Raw or heated cow milk consumption: Review of risks and benefits. *Food control*, *31*(1), 251-262.
9. Wayne, P. A. (2011). Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing.
10. EGBE, N. (2023). Antifungal effects of Nigella sativa L.(Black cumin) seed extracts and seed oil on selected Candida albicans strains. *Journal of Current Biomedical Research*, *3*(3, May-June), 993-1004.
11. Deshpande, N. M., Dhakephalkar, P. K., & Kanekar, P. P. (2001). Plasmid-mediated dimethoate degradation in Pseudomonas aeruginosa MCMB-427. *Letters in applied microbiology*, *33*(4), 275-279.
12. Enurah, L. U., Aboaba, O. O., Nwachukwu, S. C. U., & Nwosuh, C. I. (2013). Antibiotic resistant profiles of food (fresh raw milk) and environmental (abattoir effluents) isolates of Listeria monocytogenes from the six zones of Nigeria.
13. Derra, F. A. (2007). *Prevalence and antimicrobial profile of Listeria monocytogenes in retail meat and dairy products in Addis Ababa and its surrounding towns, Ethiopia* (Doctoral dissertation, Addis Ababa University).
14. Hitchins, A. D., Jinneman, K., & Chen, Y. (2022). Bacteriological Analytical Manual (BAM) Chapter 10: Detection of Listeria monocytogenes in foods and environmental samples, and enumeration of Listeria monocytogenes in foods. *Bacteriological Analytical Manual*, *23*.
15. Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American society for microbiology*, *15*(1), 1-23.
16. Hunt, K., Drummond, N., Murphy, M., Butler, F., Buckley, J., & Jordan, K. (2012). A case of

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bovine raw milk contamination with Listeria monocytogenes. *Irish veterinary journal*, *65*, 1-5.
17. Jamali, H., Chai, L. C., & Thong, K. L. (2013). Detection and isolation of Listeria spp. and Listeria monocytogenes in ready-to-eat foods with various selective culture media. *Food control*, *32*(1), 19-24.
18. Jung, Y. S., Frank, J. F., & Brackett, R. E. (2003). Evaluation of antibodies for immunomagnetic separation combined with flow cytometry detection of Listeria monocytogenes. *Journal of food protection*, *66*(7), 1283-1287.
19. Krawczyk-Balska, A., & Markiewicz, Z. (2016). The intrinsic cephalosporin resistome of Listeria monocytogenes in the context of stress response, gene regulation, pathogenesis and therapeutics. *Journal of Applied Microbiology*, *120*(2), 251-265.
20. Li, X. Z., & Nikaido, H. (2009). Efflux-mediated drug resistance in bacteria: an update. *Drugs*, *69*, 1555-1623.
21. Liu, D., Lawrence, M. L., Wiedmann, M., Gorski, L., Mandrell, R. E., Ainsworth, A. J., & Austin, F. W. (2006). Listeria monocytogenes subgroups IIIA, IIIB, and IIIC delineate genetically distinct populations with varied pathogenic potential. *Journal of Clinical Microbiology*, *44*(11), 4229-4233.
22. Liu, D., Lawrence, M. L., & Hitchins, A. D. (2008). Molecular characterization of Listeria monocytogenes strains harboring Listeria innocua putative transcriptional regulator gene lin0464. *Journal of Rapid Methods & Automation in Microbiology*, *16*(4), 412-427.
23. Luque-Sastre, L., Arroyo, C., Fox, E. M., McMahon, B. J., Bai, L. I., Li, F., & Fanning, S. (2018). Antimicrobial resistance in Listeria species. *Microbiology spectrum*, *6*(4), 10-1128.
24. Molla, B., Yilma, R., & Alemayehu, D. (2004). Listeria monocytogenes and other Listeria species in retail meat and milk products in Addis Ababa, Ethiopia. *Ethiopian Journal of Health Development*, *18*(3), 208-212.
25. Montalti, M., Soldà, G., Capodici, A., Di Valerio, Z., Gribaudo, G., La Fauci, G., ... & Gori, D. (2022). Antimicrobial Resistance (AMR) in Italy over the past five years: a systematic review. *Biologics*, *2*(2), 151-164.
26. Muhammed, W., Muleta, D., Deneke, Y., Gashaw, A., & Bitew, M. (2013). Studies on occurence of Listeria monocytogenes and other species in milk and milk products in retail market of Jimma Town, Ethiopia. *Asian Journal of Dairying & Foods Research*, *32*(1), 35-39.
27. Lee, S. H. I., Cappato, L. P., Guimarães, J. T., Balthazar, C. F., Rocha, R. S., Franco, L. T., ... & de Oliveira, C. A. F. (2019). Listeria monocytogenes in milk: occurrence and recent advances in methods for inactivation. *Beverages*, *5*(1), 14.
28. Nayak, D. N., Savalia, C. V., Kalyani, I. H., Kumar, R., & Kshirsagar, D. P. (2015). Isolation, identification, and characterization of Listeria spp. from various animal origin foods. *Veterinary world*, *8*(6), 695.
29. Osundiya, O. O., Oladele, R. O., & Oduyebo, O. O. (2013). Multiple antibiotic resistance (MAR) indices of Pseudomonas and Klebsiella species isolates in Lagos University Teaching Hospital. African Journal of Clinical and Experimental Microbiology, 14(3), 164-168.
30. Opal, S. M., Keith Jr, J. C., Palardy, J. E., & Parejo, N. (2000). Recombinant human interleukin-11 has anti-inflammatory actions yet does not exacerbate systemic Listeria infection. *The Journal of infectious diseases*, *181*(2), 754-756.
31. Poroś-Głuchowska, J. O. A. N. N. A., & Markiewicz, Z. D. Z. I. S. Ł. A. W. (2003). Antimicrobial resistance of Listeria monocytogenes. *Acta Microbiologica Polonica*, *52*(2), 113-129.
32. WC, W. (2006). Guidelines for the Collection, Transport, processing, Analysis, and Reporting of Cultures from Specific Specimens Sources. *Koneman's Color Atlas and Textbook of Diagnostic Micribiology*, 67-110.
33. Patwardhan, R. B., Dhakephalkar, P. K., Chopade, B. A., Dhavale, D. D., & Bhonde, R. R. (2018). Purification and characterization of an active principle, lawsone, responsible for the plasmid curing activity of Plumbago zeylanica root extracts. *Frontiers in Microbiology*, *9*, 2618.
34. Rousham, E. K., Unicomb, L., & Islam, M. A. (2018). Human, animal and environmental contributors to antibiotic resistance in low-resource settings: integrating behavioural, epidemiological and One Health approaches. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1876), 20180332.
35. Savadogo, A., Ouattara, C. A. T., Savadogo, P. W., Ouattara, A. S., Barro, N., & Traore, A. S. (2004). Microorganisms involved in Fulani traditional fermented milk in Burkina Faso. *Pakistan Journal of Nutrition*, *3*(2), 134-139.
36. Seyoum, E. T., Woldetsadik, D. A., Mekonen, T. K., Gezahegn, H. A., & Gebreyes, W. A. (2015). Prevalence of Listeria monocytogenes in raw bovine milk and milk products from central highlands of Ethiopia. *The Journal of Infection in Developing Countries*, *9*(11), 1204-1209.
37. Zhang, Y., Yeh, E., Hall, G., Cripe, J., Bhagwat, A. A., & Meng, J. (2007). Characterization of Listeria monocytogenes isolated from retail foods. *International Journal of Food Microbiology*, *113*(1), 47-53.

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