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Colicin production, antibacterial effect and molecular sequencing of *Escherichia coli* isolated form different sources

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Abstract

Bacteria especially *Escherichia coli* (*E. coli*) possess several mechanisms enabling them to respond to changing environment and to out compete other bacteria. *E. coli* strains are known to produce bacteriocins, one of which is colicin. Colicins are effective against closely related enterobacteriaceae and are considered viable alternatives to antibiotics. In this study Twelve (12) *E. coli* strains were isolated from food, water soil and anal swab. They were characterized, confirmed and identified as *E. coli*. The isolates were subjected to stress condition to stimulate colicin production. The colicin produced was tested against three indicator organisms namely *Salmonella* Sp, *Klebsiella* Sp and *Staphylococcus aureus.* Comparative antibiotic susceptibility test against standard antibiotics and the colicin produced was carried out to ascertain the efficacy of the colicin. *E. coli* isolates were also subjected to antibiotic sensitivity test to determine the resistance profile of the colicin producing strains of *E. coli*. Plasmid amplification to identify colicin plasmid was also carried out to determine the relationship between colicin production and resistance to antibiotics of colicinogenic *E. coli*. Result obtained showed that six out of the twelve strains of *E. coli* isolated from water produced colicin that showed high antibacterial activity against the indicator organisms. The comparative antibiotic resistance profile revealed that the colicin had better antibacterial activities than some of the antibiotics used. Furthermore the colicinogenic *E. coli* also showed resistance to some of antibiotics used. The plasmid amplification showed the colicinogenic *E. coli* possess high molecular weight (4kb) colicin plasmid while the gene sequencing revealed the presence of ColF and ColR plasmids. The result of this work underscores the fact that the use of colicin as an alternative to standard antibiotics can be further explored, especially in the search for alternatives to antibiotics considering the high level of bacteria resistance to standard antibiotics.

Keywords: Colicin; *Escherichia coli*; Indicator organisms; Enterobacteriaceae; ColF; Col R;

1. Introduction

The gut microbiota is diverse and has full arrangement of microorganisms that live in and on humans. The microbial population of the human gut is a mix of microbial genomes that contribute to the broader genetic picture, or metagenome of a human (Rogers, 2023). These microorganisms exist in a symbiotic relationship with their host, but may also become dangerous to the host as a result of antagonistic relationships between the different microbes of the micro biota.

The WHO and numerous other groups and researchers agree that the spread of AMR is an urgent issue requiring a global, coordinated action plan (WHO, 2021; CDC, 2019; Prestinaci *et al*., 2015; WHO, 2015). Many gut bacteria acquire and share genetic materials leading to resistance due to 'mobile' resistance genes.

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Bacteria especially *Escherichia coli* (*E. coli*) possess several mechanisms enabling them to respond to changing environment and to out compete other bacteria. *E. coli* strains are known to produce bacteriocins, one of which is colicin. Colicins are effective against closely related enterobacteriaceae and are considered viable alternatives to antibiotics.

Escherichia coli is the most dominant Gram negative, facultative anaerobic species in the gastrointestinal tract of warm-blooded animals, it is usually a commensal but is also implicated in a number of significant illnesses (Friedman *et al*., 2002). A part of the main mechanism of antagonistic activity of commensal *E. coli* is the secretion of colicin (Mazurek-Popczyk *et al*., 2020).

As more antibiotics are rendered ineffective by drug-resistant bacteria, focus must be shifted towards alternative therapies for treating infections. This urgent need prompts the need for efforts to investigate the efficacy of colicin against some human pathogens.

2. Material and method

2.1. Sample collection

- **Water sample**; Water samples were collected following laid down procedure as described by Marianne, (2014**).**
- **Food sample;** Ready to eat food materials were bought from vendors within the university community and transported into the laboratory for isolation of *E. coli* using the method of Cheesebrough, (2000).
- **Anal Swabs;** Samples were collected from six humans of both genders after their consents were sought. They were advised on how to collect the sample using anal swab sticks. After collection, the samples were retrieved and labelled with assigned codes.
- **Soil Sample**; Soil sample was obtained from Achievers University following laid down procedure as described by Spark *et al*., (1996). Soil samples were collected (approx 100 g) in clean, dry and sterile polythene bags using sterilized spatula and were transported to the laboratory for isolation of *E. coli*.

2.2. Isolation of *E coli.*

2.2.1. Isolation from water sample

Bacteriological analysis of *E. coli* was done according to the procedures in the US food and drug Administration-Bacteriological Analysis Manual (FDA-BAM) (1998). One milliliter of water was taken from the water sample and transferred into 9 mL of nutrient broth. It was then incubated at 37°C for 24 h under aerobic conditions. After which a loopful of the culture from nutrient broth was streaked onto EMB Agar and incubated at 37 °C for 24 h under aerobic condition. Presumptive *E. coli* colonies on LEMB Agar appear as dark centered and flat, with or without metallic sheen. Presumptive *E. coli* colonies were picked and streaked onto nutrient agar and incubated at 37°C for 24 h under aerobic condition to obtain a pure culture. Pure cultures were identified and/or confirmed using Gram staining, morphological, colonial and biochemical tests (indole production, utilization of citrate and lactose production) among others.

2.2.2. Isolation from food

A 10 g portion of food samples was pounded in a sterilized mortar and pestle. Then samples were homogenized thoroughly with 90 mL of sterile phosphate-buffered saline. (PBS; pH 7.4, Merck KGaA, Germany) solution to make a 10% sample suspension. Tenfold serial dilutions of the sample (10^{-1} to 10^{-10}) were prepared as per the recommendation of Trojan *et al*., (2016) and the International Organization for Standardization (ISO) This involved mixing 1 mL of a homogenized sample with 9 mL of sterile water. Inoculating sample was taken from the 10^{-6} dilution and pipetted into a freshly prepared MacConkey agar and EMB agar plates. The plates were then incubated at 37^0C for 24h. Thereafter, pure cultures were prepared from the plates and the pure isolates were subjected to biochemical, colonial and morphological analysis (Cheesebrough, 2000).

2.2.3. Isolation from Anal Swab

Rectal swabs were cultured as described by Schindler *et al*., (2014). Briefly, a freshly prepared agar plate of MacConkey agar was allowed to gel, Thereafter, the swab sticks collected were used to seed the surface of the gelled agar plate and the plates were allowed to incubate at 37 ^{0}C for 24 hours. After which the pure culture of the isolates was prepared using discrete colonies to seed another freshly prepared MacConkey agar plates. After incubation, the colonies were subjected to colonial, morphological and biochemical test to confirm the isolates to be *E. coli*.

2.2.4. Isolation from soil sample

The standard serial dilution technique was used for the isolation of bacteria from soil samples. One gram of soil sample was mixed with l0 ml of sterile water and serially diluted (10^{-1} to 10^{-6}) (Cheesebrough, 2000). From the serially diluted soil sample, 100 µl was mixed with warm MacConkey agar medium and poured into Petri plates. The plates were incubated at 37^0C for 24 h after which pure cultures of isolates were prepared and kept on agar slant until use.

2.3. Identification of isolates

Bacteria were identified based on colony characteristics, morphological and characteristics by Gram's staining, sugar fermentation test, methyl red, Voges-Proskauer (V-P), indole, citrate utilization, methyl red, oxidase and coagulase tests, as described by Cheesebrough, (2000).

2.4. Collection of indicator organisms

Indicator organisms; *Klebsiella* Sp, *Salmonella* Sp, *Staphylococcus aureus* and *Bacillu*s Sp were collected from the Microbiology laboratory of Department of Biological Sciences, Achievers University, Owo on agar slants and kept until use.

2.5. Screening of *E. coli* strains for colicin activity

The screening of *E. coli* strains was done using the modified method of Debroy *et al.,* (2003); and Linderberg *et al*., (2001). Briefly, *E. coli* identified strains were cultured on nutrient broth at 37^0C. At mid log phase of growth, tetracycline (0.10µg/ml), chloramphenicol (0.10µg/ml) and levofloxacin (0.10µg/ml) was added separately to each strain culture to induce stress in the *E. coli* isolates.

The broth cultures containing stressed E*. coli* strains were centrifuged to separate cell mass and supernatant. The supernatant was exposed to chloroform vapor for 30minutes while the cell mass was left untreated.

Indicator organisms were seeded in NA plates and wells measuring 6mm in diameter were made on the agar surface using a 6mm cork borer. Chloroform treated supernatants were pipetted into wells made on the surface of each agar plates already seeded with indicator organisms. The plates were allowed to stand for 1-2 hours at room temperature, and then incubated for 24hours at 37°C. Following incubation, inhibitory zones formed by antibacterial activity of supernatants were measured and compared (Askari *et al*., 2019).

2.6. Antibiotic Sensitivity

2.6.1. Antibiotic resistance status of colicigenic E. coli isolates and indicator organisms

Colicigenic *E. coli* were subjected to antibiotic sensitivity test following the guidelines of CISI (2018) to establish relationship between colicin production and antibiotic resistance of isolate The listed antibiotics were used; Taravid (10µg), Sparfloxacin (10µg), Amoxacillin (30µg), Augmentin (10µg), Levofloxacin (30µg), Tetracycline (10µg), Gentamycin (30µg), Ciprofloxacin (30µg) Chloramphenicol (30µg), and Septrin (30µg). Briefly, *E. coli* isolates were grown in nutrient broth for 24hours at 37°C, after incubation *E. coli* isolates were transferred into test tubes containing 5ml of 0.85% sterile physiological saline. The turbidity of the suspension was adjusted to 0.5 McFarland standards (Equivalent to 1.5×10^8 CFU/100ml) sterile swabs were soaked with the bacterial suspensions and spread on the surface of Muller Hinton agar, after which antibiotic discs were placed on the agar surface and incubated at 37°C for 24hours. After incubation, the plates were examined for zones of inhibition which were measured and recorded as susceptible or resistant and interpreted using the zone diameter interpretation according to CLSI guideline (2018). This procedure was also repeated for the indicator organisms.

2.7. MARI of Colicigenic *E. coli* and Indicator organisms

The multiple antibiotic resistance indices of the colicigenic *E. coli* and indicator organism were determined. MAR index is calculated as the ratio between the number of antibiotics that an isolate is resistant to and the total number of antibiotics the organism is exposed to. A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used (Rotchel and Paul, 2016).

2.8. Molecular Identification of The Isolates

2.8.1. DNA Extraction

The DNA of *E. coli* isolates was extracted by suspending colonies from the overnight culture of each isolate on Nutrient agar plates into 100 µL 1X Tris-EDTA buffer, vortexed and boiled at 100°C for 10 minutes as described by Adesiyan *et al*., (2019). The boilate was transferred immediately to the freezer (-20°C) for 10 minutes. The resulting supernatant containing DNA of each isolate was collected, stored at 4°C and used as a DNA template for PCR analysis.

2.8.2. Molecular Identification of E. coli through PCR Amplification of 16s rRNA Gene

All organisms assumed to be *E. coli* by their phenotypic and macroscopic characteristics were confirmed as *E. coli* by amplifying their 16S rRNA gene (Hassan *et al*., 2014). *E. coli* strain ATCC 25922 was used as the positive control while sterile distilled water was used as the negative control. A 12.5 µL reaction mixture contained 6.25 µl of One Taq Quick-Load 2XMaster mix with Standard Buffer (Bio Labs, New England), 0.25 pmol each of the forward and reverse primers (Inqaba, Biotec, South Africa), 2µl of the DNA template and made up with 3.75 µl nuclease free water (BioConcept, Switzerland). Amplication conditions were as follows: Initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 94°C for 45s, annealing at 45°C for 45s, and extension at 7°C for 1 min; followed by a final extension at 72°C for 5min. Each amplicon (10 µl) was electrophoresed on a 1.5% agarose gel (Cleaver Scientific, United Kingdom) pre stained with 0.5µg/ml Ethidium bromide in 1X Tris-Acetate-EDTA(TAE) buffer and viewed with a UVitec transilluminator (Avebury, Cambridge UK).

2.9. Plasmid Isolation from Colicinogenic *E.coli*

Plasmids were isolated using the QIAGEN Plasmid Purification mini kit.

2.9.1. Gel Integrity

The integrity of the extracted plasmid was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 10µl of each PCR product and loaded into the wells after the 1KB DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of the molecular weight ladder that was ran alongside experimental samples in the gel (Sambrook *et al*., 1987).

2.10. Primer Design

In order to design specific primers to the specific Colicin gene complex genes, *Escherichia coli* str. K-12 of accession number NC_000913.3 obtained from the gene bank database on NCBI website (National Center for Biotechnology Information) was used as the reference sequences and the colicin V production protein was mapped out and located at position 2430275 to 2430763 of the stain genome. https://www.idtdna.com/PrimerQuest/Home/Index site was then accessed and sequence pasted in the sequence entry box and multiple PCR primers were generated. It is very necessary to ensure that the primers will have a perfect match, this will enhance primer annealing during PCR. To do this, primers must anneal to regions where the sequences are conserved. Each primer pair was then checked for specificity to be sensitive to only the genes of interest to which it was designed to detect and also ability to cut across all aligned genes then the best primer was selected and synthesized at Inqaba in South Africa.

2.11. PCR

The PCR preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each ColF-GTCTGGTTTACGGGCTTTGA and ColR – CGGTAAAGGAGTCGAGAAAGAAG primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a Pcr profile consisting of an initial denaturation at 94°C for 5 min; followed by a 35 cycles consisting of 94°C for 30 s, 50°C for 30s and 72°C for 30 seconds; and a final termination at 72°C for 10 mins. And chill at 4oC.

2.12. Data Analysis

Data derived was grouped in variables, presented in tables and charts and analyzed using statistical packages SPSS, Excel office software and further data analysis tool(s).

3. Results

3.1. Identification of isolates.

Twelve (12) of the bacteria isolated from water, food, soil and anal swab were identified as *E. coli* via their colonial, morphological, and biochemical characteristics.

3.2. Colicin production by *E. coli* isolates

Out of the twelve (12) *E. coli* strains identified**,** only five of them were able to produce colicin. The antimicrobial activity against the indicator organisms confirming colicin production is presented in table 1.

Strain from Culture containing *E. coli* strain E3 showed antibacterial activity against indicator organisms with zones of inhibition as indicated. *Klebsiella* Spp (32 mm), *Staphylococcus aureus* (38 mm) and *Salmonella* Sp (34 mm). E5 also showed zones of inhibition against indicator organisms as indicated. *Klebsiella* Sp (18 mm), *Staphylococcus aureus* (17 mm) and *Salmonella* Sp (17 mm). For E6, *Klebsiella* Sp had zone of inhibition of 20mm, *Staphylococcus aureus* (21mm) and *Salmonella* Sp (16mm)**,** AC1 showed zone of inhibition of 20mm against *Salmonella* Sp, 25mm against *Klebsiella* Sp and 24mm against *Staphylococcus aureus* while AM 2 showed a zone of inhibition of 19mmagainst *Salmonella* Sp, 20mm against *Klebsiella* Sp and 18mm against *Staphylococcus aureus.*

Table 1 Colicin production by *E. coli* and activity against indicator organisms

	Zones of inhibition (mm).		
Isolates	***Salmonella* Sp**	***Klebsiella* Sp**	***Staphylococcus aureus***
E1 (Anal swab)	20	25	18
E2 (Anal swab)	19	20	18
E3 (Food)	34	32	38
E5 (Food)	17	18	17
E6 (Food)	16	20	24
E7 (food)	R	R	R
E8 (water)	R	R	R
E9 (water)	R	R	R
E10 (Soil)	R	R	R
E11 (soil)	R	R	R
E12 (soil)	R	R	R

3.3. Comparative Susceptibility of Indicator Organisms to Standard Antibiotics and Colicin.

The results for the susceptibility test of the indicator organisms to standard antibiotics and colicin produced by *E. coli* strains are presented in Table 2. All indicator organisms were sensitive to colicin, with zones of inhibition ranging from 15 mm (E2) – 38mm (E3). whereas *Salmonella* sp was resistant to septrin, sparfloxacin, amoxicillin, augmentin, amplicox, zinnacef, rocephin, erythromycin and gentamycin. *Klebsiella* Sp was resistant to ciprofloxacin, amplicox, zinnacef, rocephin and erythromycin but sensitive to other antibiotic. *Staphylococcus aureus* was resistant to chloramphenicol, sparfloxacin, augmentin and taravid.

Table 2 Comparative Sensitivity of indicator organisms to antibiotics and colicin

	Zones of inhibition in mm		
Antibiotics	***Salmonella Sp***	***Klebsiella Spp***	***Staphylococcus aureus***
Septrin (30µg)	R	R	30
Chloramphenicol (30 µg)	24	21	R
Sparfloxacin (10 µg)	R	21	R
Ciprofloxacin (30 µg)	30	R	30
Amoxacillin (30 µg)	R	11	20
Augmentin (10 µg)	R	15	R
Gentamycin (30 µg)	R	16	20
Pefloxacin (10 µg)	25	20	30
Taravid (10 µg)	26	22	R
Streptomycin (30 µg)	23	23	30
Ampiclox (30 µg)	R	R	27
Zinnacef (20 µg)	R	R	27
Rocephin (25 µg)	R	R	28
Erythromycin (10 µg)	R	R	30
	COLICIN		
E1	20	25	18
E2	19	20	18
E3	34	32	38
E4	17	18	17
E5	16	20	24

3.4. Sensitivity of colicigenic *E. coli* to antibiotics

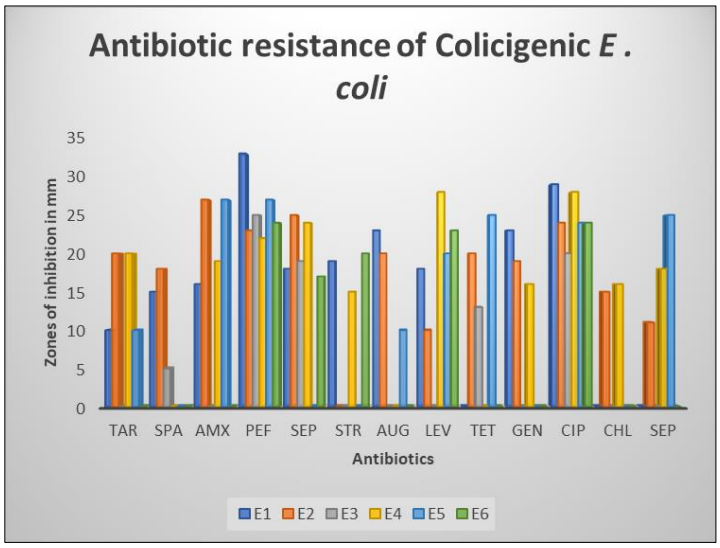

Figure 1 Antibiotic resistance of Colcigenic *E. coli* to antibiotics

The colicgenic *E. coli* strains were subjected to sensitivity test to antibiotics and the result is as presented in Fig 1. E1 was resistant to 23% of the antibiotics, E2 was resistant to only 8% of the antibiotics used (taravid), E3 was resistant to 62% of the antibiotics, E4 was resistant to 23% antibiotics, E5 was resistant to 38% antibiotics and E6 was resistant to 62% antibiotics.

3.5. Multiple antibiotic resistance index

Salmonella Sp had MARi of 0.64, *Klebsiella* had MARi of 0.36 and *Staphylococcus aureus* had MARi of 0.29 as depicted in table 3.

Table 3 Multiple antibiotic indices of indicator organisms

Organisms	No of antibiotic used	Number of antibiotics organisms are resistant to	MARI
Salmonella Sp	14	9	0.64
Klebsiella Sp	14	5	0.36
Staphylococcus aureus	14	4	0.29

3.6. Statistical analysis of data

The statistical analysis was carried out using the SPSS (Statistical Package for the Social Sciences). From the result, there are significant differences in the values of the resistance of colicigenic *E. coli* isolates to standard antibiotics and the non-colicigenic isolates (P> 0.05) at 50% confidence interval. In the test for sensitivity of indicator organisms to standard antibiotics and colicin, there are significant differences in the values obtained when comparing the efficacy of colicin against indicator organisms as compared to antibiotics ((P> 0.05) at 50% confidence interval.

3.7. Plasmid status of colicigenic *E. coli*

Two colicgenic *E. coli* were subjected to plasmid amplification to identify the col plasmid in the gene. EI and E3 both have the positive amplification of the colicin gene complex using gene specific primers of 230 kbp and Col plasmid of approximately 4 kbp respectively (Plates 1-4).

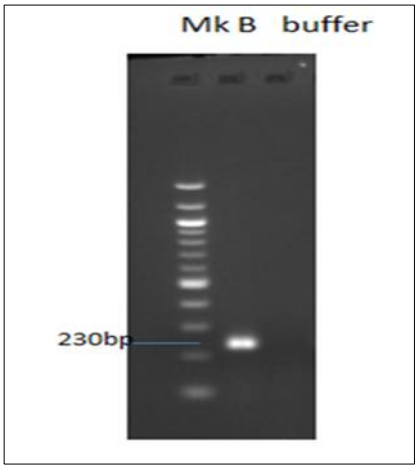

Plate 1

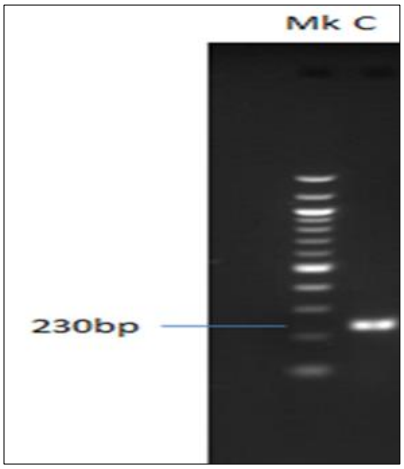

Plate 2

Figures 2 and 3 Gel electropherogram indicating the positive amplification of the colicin gene complex using gene specific primers. A band size of approximately 230bp indicates a positive amplification

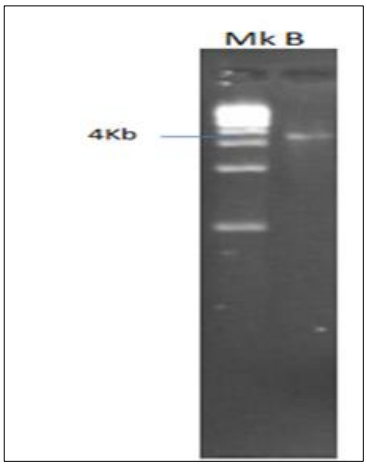

Plate 3

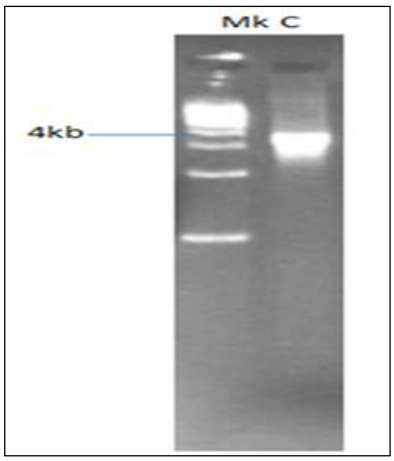

Plate 4

Figures 4 and 5 Gel electropherogram indicating the presence of high molecular weight plasmids of approximately 4KB

3.8. Gene sequencing of colicigenic *E. coli*

Gene sequencing for the colicin plasmid primer in E3 are . ColF- GTCTGGTTTACGGGCTTTGA and ColR– CGGTAAAGGAGTCGAGAAAGAAG while gene sequencing for E3 were ColF-GTCTGGTTTACGGGCTTTGA and ColR – CGGTAAAGGAGTCGAGAAAGAAG.

4. Discussion

Colicins belong to a class of bacteriocins and are antimicrobial proteins produced by *Escherichia coli* that are effective against closely related enterobacteriaceae (Mader *et al.,* 2015). Colicin is a suitable alternative to antibiotics that are currently not active against bacteria. *E. coli*, a Gram negative, facultative anaerobic, rod shaped, coliform bacterium of the genus Escherichia, is commonly found in the lower intestine of warm-blooded organisms (Tenaillon *et al.,* 2010).

Several strains of *E. coli* are known to produce different types of bacteriocin. Gordon and O'Brien (2006) recorded the presence of colicinogenic genes in *E. coli* isolated from environment and animal specimen.

In this study, *E coli* isolated from different sources produced colicin with different degrees of antibacterial activity but isolates from food and anal swabs (E1 and E3) had higher efficacy against the indicator organisms (tables 1 & 2). The ability to produce bacteriocins (bacteriocinogeny) is common to and more than half of *E. coli* strains isolated from human fecal microbiota produce at least one bacteriocin type (Bosak *et al*., 2021). Similarly, Drissi *et al*. (2015) suggested that bacteriocins producing *E. coli* strains are widespread across the human gastrointestinal tract. Several authors have been able to identify several colicin producing *E. coli* strains (Cascales *et al*, 2007; Jakes, 2012). Budic, *et al* (2011) recorded high efficacy of colicin against test organisms when in combination with other types of colicin, he however discovered high level of resistance to colicin by *E. coli* strains isolated from patients with bacteremia. Mazurek-Popczyk *et al* (2020) reported that the colicin produced by commensal *E. coli* was active against zoonotic *E. coli*. This is in support of the result obtained in this work where the colicigenic *E coli* isolates had high antibacterial activity against the indicator organisms used in this study.

The efficacy of colicin against *Staphylococcus aureus* is interesting because bacteriocins are known to work against closely related species. *Staphylococcus aureus* are Gram-positive cocci that are commensals of skin and mucous membrane of healthy individual and implicated in diseases ranging from skin or soft tissue infections to systemic and fatal diseases (Tong *et al*., 2015). Dabandi *et al*., (2022) reported that bacteriocins produced by lactic acid bacteria was effective against foodborne pathogens such as *Staphylococcus aureus, Pseudomonas fluorescens, P. aeruginosa, Salmonella typhi, Shigella flexneri, Listeria monocytogenes, Escherichia coli* O157:H7, and *Clostridium botulinum*. Etayash, *et al*., (2015) also posited that bacteriocin can have broad spectrum activity against wide range of related or unrelated species. Further investigation into using bacteriocin against other species of bacteria could be explored in the future.

The comparative antibiotic sensitivity test of indicator organisms showed that all organisms were sensitive to colicin while they were resistant to some of the antibiotics used (Table 2). Several authors have recorded high level of resistance of the indicator organisms used in this work to antibiotics (Applebaum, 2007; Miller *et al*., 2011; Hur *et al*., 2012; Lauteri *et al.,* 2020; Deyno *et al.*, 2021; Temikotan and Daniels, 2022).

Some colicin producing *E. coli* strains have been known to have resistance to some standard antibiotics, In this work the colicin producing *E. coli* strains were found to have resistance to some antibiotics used, Fig 1 shows that E1 was resistant to two antibiotics, E2 was resistant to one antibiotic, E3 was resistant to eight antibiotics, E4 was resistant to three antibiotics, E5 was resistant to three antibiotics, E6 was resistant to seven antibiotics. In corroboration, Djonne (1985) observed resistance of colicin producing strains of *E. coli* to tetracycline, neomycin and ampicillin than among non-colicin producing strains. Riley & Gordon (1996) also reported high level of resistance of colicin producing *E. coli* to antibiotics, several studies have supported the fact that some *E. coli* strains can carry both the colicin production and antibiotic resistance gene (Feldargen & Riley 1988).

Figures 1 & 2 depicts the positive amplification of the colicin gene plasmid complex in colicigenic *E. coli*, (E1 and E3) with band sizes of approximately 230bp. This is an evidence that the colicin production characteristic is plasmid borne and the plasmids were identified as Col-plasmids with molecular weight of 4kb each (Figures 3 & 4). In a previous study, Inou *et al*., (1991) isolated Col E1 plasmid from *E. coli* with 6.6. kb size.

Summarily colicins are considered viable alternatives to antibiotics as a result of the mechanism of action which include; disruption of cellular components that prevents the bacteria growth, which differs from inhibition of protein synthesis carried out by some antibiotics like canamycin, cell wall synthesis by penicillin and DNA replication by ciprofloxacin (Walsh 2000). Secondly colicin may not be toxic to humans as their cytotoxicity is only on bacteria that produce receptor proteins which are not present in human cells (Cascales *et al*., 2007). Thirdly the cell killing kinetics of colicin are fast which may eradicate harmful bacteria during the log phase of growth thereby preventing development of resistance (Jakes 2012).

The amino acid sequence (ColF- GTCTGGTTTACGGGCTTTGA and ColR– CGGTAAAGGAGTCGAGAAAGAAG) identify the colicin gene to be ColF and ColR. ColR is noted for its pore forming activity and its production in biofilms formed by *E. coli* isolates. Rendueles *et al*., (2013) reported that Colicin R displaced increased activity against *E. coli* strains that have a reduced lipopolysaccharide length such as the pathogenic enteroaggregative *E. coli*. He further clarified that the size of lipopolysaccharide is an important determinant for resistance to colicin.

5. Conclusion

Harnessing the potential of colicins, while considering the associated risks, offers promise for combating antibiotic-resistant pathogens and advancing the field of antimicrobial therapeutics. It should be of note that further research into the advantages of bacteriocins over antibiotics be encouraged in order to be able develop effective solutions to this pressing global health challenge of bacterial resistance.

The identification of colicin-producing *E. coli* strains and their association with antibiotic resistance is of paramount importance. The observed positive correlation between colicin production and antibiotic resistance suggests that colicins could be utilized as natural alternatives to traditional antibiotics. Additionally, the exploration of plasmid-mediated gene transfer adds depth to our understanding of the dissemination of colicin genes and antibiotic resistance. This study's findings have direct implications for public health.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained from the Ethical Review committee of the Directorate of Research, innovation and Linkages of Achievers University, Owo, Ondo State, Nigeria.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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