World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-15, October 2022
ISSN: 2749-3644

STUDY THE EFFECTIVENESS OF MODIFIED LEVOFLOXACIN (MOLE) ON GRAM NEGATIVE BACTERIA ISOLATED FROM CHILDREN STOOL

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Article history:

Received: August 20th 2022
Accepted: September20th 2022
Published: October 26th 2022

Abstract:

INTRODUCTION:

It is a medication available as tablet, solution, and fluid formulations for the eyes (eye drop). It is commercially available as tablets, oral solutions, and injections. It is rapidly and completely absorbed following oral administration, with the same plasma absorption profile observed following intravenous administration for the same duration. [1-2]. LF has broad antimicrobial activity against numerous gram-positive and gram-negative bacteria. It functions by inhibiting bacterial topoisomerase IV and DNA gyrase, enzymes required for DNA replication, repair, transcription, and recombination. [2].

LF Overdose symptoms include disorientation, dizziness, slurred speech, drowsiness, nausea, vomiting, muscle issues, nerve damage, behavioral changes, or severe mood changes or hypoglycemia. Rarely, LF can cause aorta damage, which can result in life-threatening bleeding or death. [3].

Campylobacter jejuni is the leading cause of gastroenteritis in children and adults. It can occasionally cause systemic infection in children and the elderly. They are gram-negative bacteria, bacilli, and fussy bacteria that require a nutrient-rich medium, are microaerophiolic, and require high CO2 levels. Campylobacter jejuni has a generation time (GT) of between 48 and 72 hours [4]. In infants and young children with compromised immune systems, infections of the stomach and intestines, bareraemia, meningitis, response arthritis, Tpitis, Sepsis (infection in patients with HLA B27), Guillain-Barre syndrome, endocarditis, and inflammatrybwel Miller-Fisher disorder are significantly more severe. [5]. Due to the self-limiting nature of campylobacteriosis, fluoroquinolone or macrolide antibiotics are typically prescribed to patients with persistent symptoms. [6]. However, Campylobacter is becoming more resistant to these essential antibiotics, specifically fluoroquinolones. [7]. As a result, resistance to two essential anti-Campylobacter drugs, ciprofloxacin (fluoroquinolone) and azithromycin (macrolide), has increased, resulting in 310,000 untreatable infections and 28 annual deaths in the United States. [8].

Keywords: Eye drop, Campylobacter drugs, ciprofloxacin (fluoroquinolone) and azithromycin (macrolide),

MATERIALS AND METHODS

Bacterial isolation :

During the period 2021-2022, 100 stool samples of children suffering from diarrhea are included in this study for bacterial culture using specific Campylobacter agar base (PRESTON) under microaerophilic conditions and confirmation by molecular study. Patients range in age from 2 months to 10 years, with different sexes.

Preparation of Modified levofloxacin (MOL):

Modified levofloxacin (MOL), composed from (levofloxacin, bathophen- anthroline (BPhen), and copper chloride dihydrate) [Cu(lvx)(BPhen)Cl]+, (C42H37Cl2CuFN5O4).

MOL was diluted to provide these concentrations: 10mg/ml,20mg/ml,30mg/ml,40mg/ml,50 mg/ml and60mg/ml using DMSO solution (Sigma Aldrich, Germany).

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Each concentration was applied to bacterial culture (broth) in a total count of $1X10^5$ C.F.U./ml and incubated for 3 hrs at 37˚C.

Cell lysis:

About 1.5 ml of the treated cultures of *Campylobacter spp*. were collected and centrifuged (12000 *xg* for 2 minutes) and then the pellets were re-suspended and lysed using lysis buffer (50 mMTris-HCl, 20 mM $MgCl_2$, 30% w/v raffinose).

Modified levofloxacin (complex)

5mL of a methanolic solution of CuCl2.2H2O (0.2mmol, 34mg) was added drop-wise to 10mL of deprotonated lvx (72mg, 0.2mol) and stirred for 30 minutes at room temperature. The solution mixture was treated with a 10mL methanolic solution of BPhen (66 mg, 0.2 mmol) (C24H16N2) and refluxed at 601C for 2 hours. The solution was then filtered and allowed to dry. After 4–5 days, dark green crystals were obtained and analyzed by (FTIR)analysis. 125mg,79%. M.P.:298–300 1C. Analcalc for [Cu(lvx)(BPhen)Cl]. Cl, molecular weight(833.23): C,60.83; H,4.50;N,8.45%. C=60.71; H=4.36; N=8.18%.

SDS-PAGE (Polyacrylamide gel)

Samples were mixed with 3XLaemmli loading sample buffer (187.5 mMTris-HCl,pH6.8, 6% (v/v) SDS, 30% (v/v) glycerol,300mM DTT,0.625 %(v/v) bromophenol blue). The samples were separated using 4%stacking gel and 15%separated gel (Stacking gel;250 mMTris-HCl,pH6.8,0.1%(v/v)SDS,4% acrylamide(37.5:1 acrylamide:bis-acrylamide),0.06% (w/v) APS,0.015 % (v/v) TEMED).

Resolving gel; 750 mMTris-HCl, pH8.9, 0.1%(v/v) SDS, 8%,10% or 15% acrylamide* (37.5:1 acrylamide:bis-acrylamide), 0.06 % (w/v) ammonium persulfate (APS), 0.015 % (v/v) TEMED. Samples (14L of sample with 7L of Laemmli loading sample buffer) were run at 100voltage for 20minutes, then 200voltage for 40minutes. Gels washed with distilled water then stained with Coomassie brilliant blue (0.1% (w/v) Coomassie brilliant blue, 50% (v/v) methanol, 7% (v/v) glacial acetic acid). The gels were destained with methanol and glacial acetic acid (20% v/v).

RESULTS AND DISCUSSION:

Isolation and Identification of *Campylobacter sp.*:

The results show that the positive isolated of Campylobacter sp. from patient was 8:100 (8 %) and 92:100 (92%) was negative results as show in figure 1.

Confirm isolated was done by using PCR based on specific primers 23S rRNA &16S rRNAwith molecular length about650 and 402bp respectively. This result agree with Kadhim et al., 2018 [9]. As show in (figure 2).

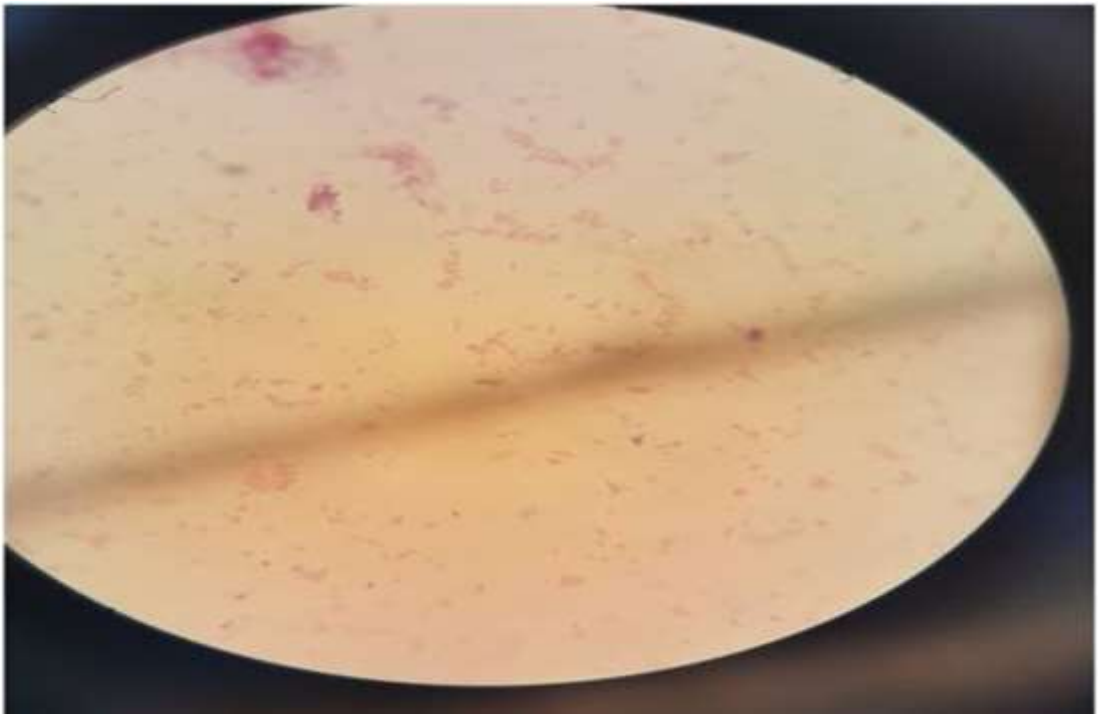

Figure 1. Campylobacter sp under microscope

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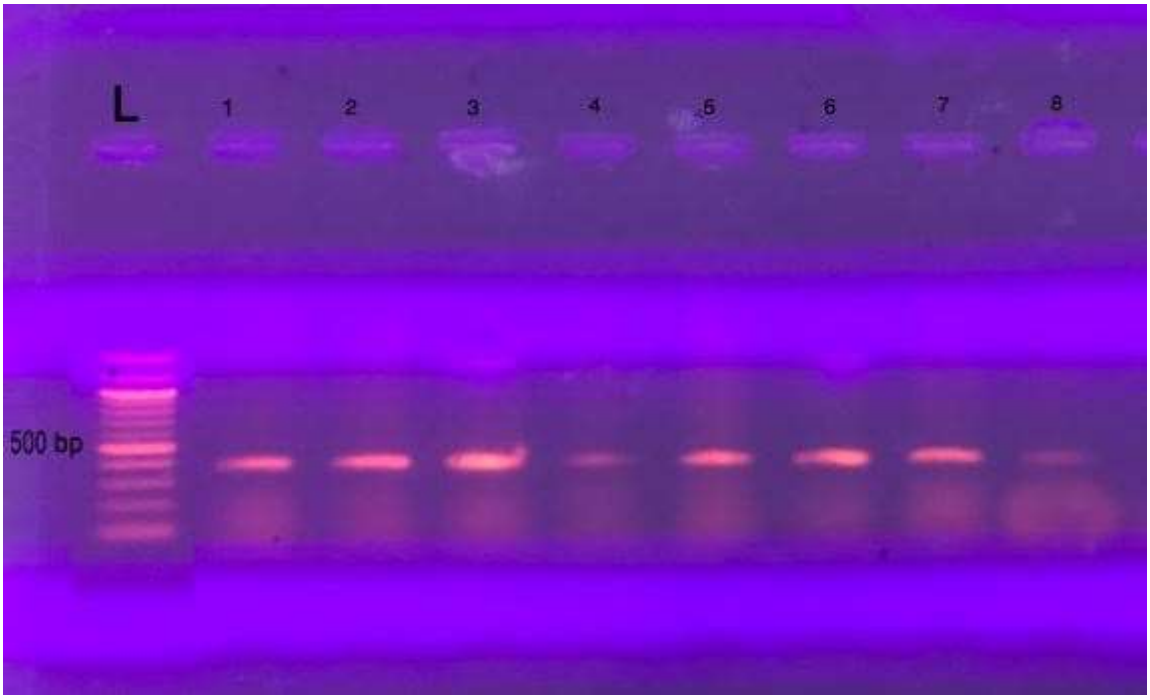

Figure 2 Agarose gel electrophoresis for 16SrRNA PCR products,gel was electrophoresed for 1hours at 70Volt. size of PCR product is 402bp.

Nucleic acid-for diagnostics presentsigneficant sensitivity, and can be determine for both the presence also infection, so differentiate between Campy lobacter contagions at the species level. Thus promotePCR, if possible, as the detection of Campylobactersp. for epidemiologicof studies to the. This protien will made the fullest ascertainments of the bearing of Campylo bacter infections [10].

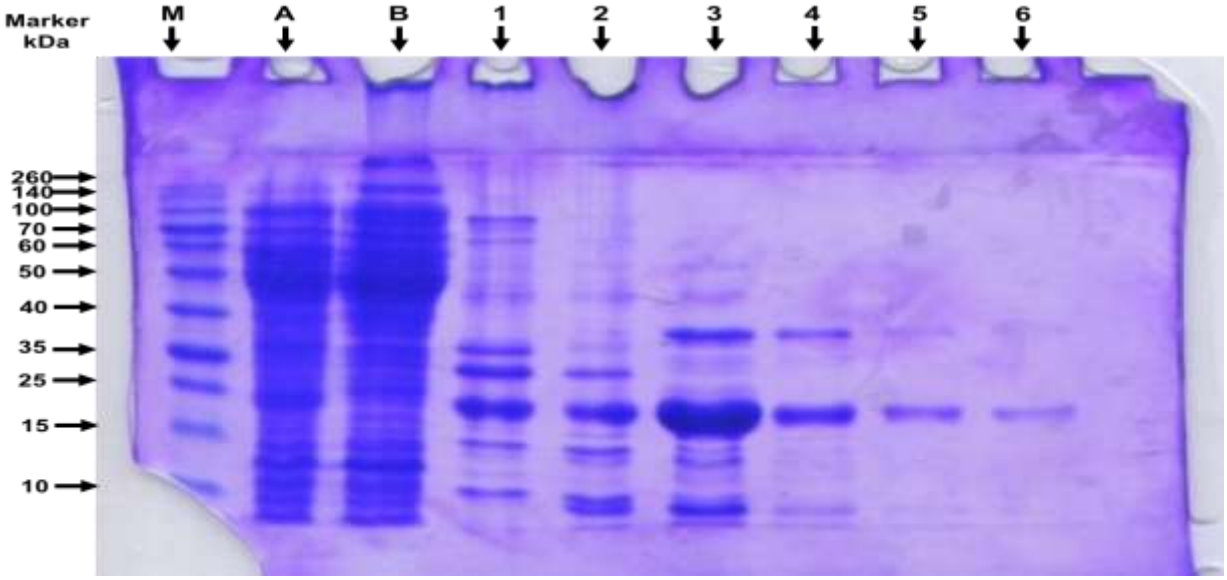

Figure 3: SDS-PAGE separation gel of the effect of Modified levofloxacin (MOL) *on Campylobacter spp*. proteins expression and translation

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M: Marker (Spectra™ MulticolorBroad Range ProteinLadder, ThermoScientific™, Germany); A: *Campylobacter spp.* culture without MOL, B: *Campylobacter spp.* with DMSO only; 1: *Campylobacter spp.* treated with 10 mg/ml of MOL; 2: *Campylobacter spp.* treated with 20 mg/ml of MOL; 3: *Campylobacter spp.* treated with 30 mg/ml of MOL; 4: *Campylobacter spp.* treated with 40 mg/ml of MOL; 5: *Campylobacter spp.* treated with 50 mg/ml of MOL; 6: *Campylobacter spp.* treated with 60 mg/ml of MOL. About 20 microliters of each samples were loaded per well. Coomassie Blue staining was performed.

DISCUSSION

The results of (14) and (15) showed that the partitioning of natural proteins on polyacrylamide gels needs to be based on charge in addition to atomic size. For a few protein atoms, dodecyl sulfate particles are limited to proteins 41, Due to this, one must be prepared for the unique burden of each protein by limiting SDS, which makes all particles negatively charged. Currently, it's hard why proteins amino acids and isoelectric concentrations collect according to the basic plan. The screening impact may win out over the marginal heap impact. Due to theoretical difficulties, we'll only look at functional results. Two issues raised. This exam. How consistent are Reproducible (SDS gel electrophoresis) results and sub-atomic loads? findings are clear. Show beyond a reasonable doubt that capillary electrophoresis . Only the subatomic loads of their polypeptide chains appear to control the isoelectric point and amino acid decay. We after discovering that nearly 40 protein molecules have electrophoresis portability that is not dependent on. polypeptic chains used in corrosive amino-sequence tests have known subatomic loads. Several competing polypeptide chain length characteristics have been documented (15). Gel filtration showed 10,000-260,000 (Figure 3). The SDS electrophoresis generates 15,000 fragments. According to all of the evidence, for various proteins, we believe that the two characteristics demonstrating 15,000 are vastly superior to the one demonstrating 10,000 between the degree of clustering of MOL between 10 and 60 mg/ml as revealed by Fonda and Anderson (16). Their value is derived primarily from a unique mark analysis and a gelfiltration study, the latter of which is less comprehensive than Henn and Ackers' study . Currently, the method appears to yield values that coincide with the best current estimates for each of the considered proteins; thus, it seems reasonable to compare (SDSgel) electro. with other methods. It has been demonstrated that freezes possess an extraordinary settling force over a vast number of subatomic loads. In this regard, the method represents a substantial improvement over gel filtration models performed on Sephadex in a denaturing dissolvable medium (17). The excellent outcome, the fact that a measurement of the subatomic in something like a day, and the relatively that is required place this method in direct competition with other commonly used techniques. It is in no way consistent with the assumption that the precision of gel practically identical to advanced physic o-substance techniques. A few hypothetical highlights of SDS gel electrophoresis are accurate as well., and despite the, as well as the results presented above, a case can be made for its wider application, particularly in the case of conflicting information obtained from different methods.

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Volume-15, October 2022
ISSN: 2749-3644

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