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Molecular Characterization of Multi-drug Resistant *Streptococcus pyogenes* and Its Eradication Using Essential Oil of *Zingiber officinale* as a Natural Antimicrobial Agent

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MULTIDRUG-resistant (MDR) *Streptococcus pyogenes* is implicated in a variety of diseases. *S. pyogenes* has been found to produce novel enzymes to break down antibiotics more than most other bacteria. This study aimed to detect and to identify *S. pyogenes* in some clinical samples by serotyping method especially multidrug resistant *S. pyogenes* with detection and sequencing of *meF* and *ermB* genes responsible for Macrolide resistance, *parC* gene responsible for Quinelone resistance and *folP* gene responsible for Sulfonamide resistance and using essential oil of *Ginger (Zingiber officinale)* as a natural treatment agent for MDR *S. pyogenes* strains. *S. pyogenes* was detected in 60/120 throat and blood samples (50.0%). 36/60 samples were found to be multidrug resistant. Men were more likely infected than women. The results indicated that 52.8% of *S. pyogenes* isolates harbored *meF* gene, 72.2% harbored *ermB* gene, 100.0% harbored *parC* gene and 100.0% harbored *folP* gene while 25% of them harbored the four genes. The sequencing alignment of *meF, ermB, parC* and *folP* genes showed some mutations in the four genes ranged between inversion mutation in *meF* gene, substitution mutation in *ermB* and *parC* genes and deletion and inversion mutations in *folP* gene. *Zingiber officinale* essential oil was analysed by GC-MS which illustrated that 17-Octadecynoic acid was the most common ingredient with percentage of 48.27%. 20µg/ml was found to be the minimal bactericidal concentration (MBC).

Keywords: Multi-drug resistant, *S. pyogenes, Zingiber officinale.*

Introduction:

Streptococcus pyogenes (group A *Streptococcus*, GAS) is a gram-positive bacterium that is one of the top ten causes of infectious illness death globally, accounting for more than 517,000 deaths per year. GAS causes a wide range of symptoms, from minor pharyngitis to life-threatening invasive infections (Walker et al., 2014).

Direct contact, infected fomites, foodborne contamination, or droplets from those with pharyngeal infection or colonisation can all spread *S. pyogenes* (Makthal et al., 2019). Penicillin was used to treat most *S. pyogenes* infections, and it is still effective for emergency therapy (Shulman et al., 2012). Patients who were allergic to penicillin were given erythromycin, amoxicillin, cotrimoxazole, chloramphenicol, tetracycline, azithromycin, and clindamycin instead (Behnamfar et al., 2019). As a result of needless antibiotic exposure and drug resistance, current treatment recommendations prohibit the empirical use of antibiotics (Luo et al., 2019).

Antibiotic resistance is linked to an increase in mortality, which has become a global public health concern (Silva-Costa et al., 2015).

Target site modification is mediated by erythromycin rRNA methylases, predominantly *ermB*, which methylate the 23S rRNA and block antibiotic binding to the ribosome. This modification provides resistance to macrolides, lincosamides,

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and streptogramin B and confers the Macrolide-Lincosamide Streptogramin B (MLSB) resistance phenotype. Although the macrolide efflux activity was initially attributed to *mefA* (Silva-Costa et al., 2015). Intrinsic reduced susceptibility to fluoroquinolones due to a polymorphism in the quinolone resistance determining region (QRDR) of the *parC* gene. Analysis of the parC gene sequence of some of those fluoroquinolone-resistant isolates has demonstrated point mutations along the QRDRs analogous to previously reported mutations in *S. pyogenes* (Orscheln et al., 2005).

Ginger (Zingiber officinale) is one of the most commonly utilised plants (Wang et al., 2017). *Z. officinale* has been used in the treatment of indigestion, flatulence, constipation, and nausea, as well as headaches, rheumatism, colds, and coughs (Grzanna et al., 2005). *Z. officinale* has been intensively explored for its therapeutic characteristics using advanced scientific techniques in recent decades, and a range of bioactive chemicals have been extracted from various areas of the plant. Antimicrobial activity, anticancer activity, antioxidant activity, antidiabetic activity, nephroprotective activity, hepatoprotective activity, larvicidal activity, analgesic action, anti-inflammatory activity, and immunomodulatory activity have all been reported for the plant (Ho et al., 2013).

This study aimed to isolate and identify multi-drug resistant *S. pyogenes* strains in some clinical isolates from Egypt and detect specific genes involved in various drug resistances, as well as their sequences. So, it has examined the effects of different concentrations of essential oil of *Ginger (Zingiber officinale)* to prevent and control MDR *S. pyogenes* strains.

Materials and Methods:

Sample collection

120 clinical sputum and blood specimens were collected from Dar Al-Fouad Hospital (60 samples), 6th October Hospital (20 samples) and Al-Borg Laboratories (40 samples) during April 2018 to January 2020. Patient's age and gender was recorded. Subsequently, standard techniques were used to process the samples (Baveja, 2012).

Bacterial isolation and identification

Bacteria was isolated, identified and confirmed by VITEK 2 system version 9.02 (BioMerieux, USA) (Henning et al., 2015). Serotyping method (LK06-HiStrepTM Latex Test Kit, India) was used to identify *Streptococcus spp,* especially *S. pyogenes***.**

Antibiotic susceptibility test (disc diffusion)

The antibiotic susceptibility test of the isolated *S. pyogenes* strains was evaluated against twelve different types of antibiotics from nine distinct groups, (Penicillins (Ampicillin 10µg), Carbapenems (Meropenem 10µg), Macrolides (Azithromycin 15µg), Cephalosporins (Cefaclor 30µg, Cefoperazone 75µg, and Cefepime 30µg), Quinolones (Ciprofloxacin 5µg and Nalidixic acid 30µg), Sulfonamides (Trimethoprim/ Sulphamathoxazole 1.25/23.7µg), Phenicols (Chloramphenicol 30µg), Cyclines (Doxycycline 5µg) and Aminoglycosides (Gentamycin 10µg)) (Oxoid, England)

Antibiotic discs were first placed on Muller-Hinton agar plates inoculated with 0.5 McFarland inoculum overnight grown. Then, the plates were incubated for 24h at 37°C. Subsequently, the diameter of the inhibition zone was measured and compared to Clinical & Laboratory Standards Institute (CLSI) 2014 guidelines (Freeman et al., 2014).

DNA extraction of S. pyogenes

The QIAamp DNA Mini Kit was used to extract total bacterial DNA (QIAGEN, USA). *S. pyogenes* was newly cultivated on 5ml nutrient broth medium at 37°C for 24h. After centrifugation for 10min at 7500rpm, the supernatant got discarded. Next, the pellet was resuspended in 180µL of lysis buffer and incubated for 30min at 37°C. 200µL of Lysis Solution was mixed with 20µL of Proteinase K, and the sample was incubated at 70°C for 10min using a shaking water bath. Once 200µL of 100% ethanol was added, all microcentrifuge tubes were transferred to a spin column, centrifuged for 1min, and the filtrate tube discarded. Finally, 200µL AE (elution buffer) was added, incubated at room temperature for 1min, and centrifuged for 1min. The extracted DNA was kept at -20°C until PCR was carried out.

Detection of meF, ermB, parC, and folP genes

PCR analysis was used to detect the *meF* and *ermB* genes responsible for Macrolide resistance, *parC* gene responsible for Quinelone resistance, and *folP* gene responsible for Sulfonamide resistance in the identified MDR *S. pyogenes* isolates (Table 1).

TABLE 1. The sequences of the specific primers used to detect *meF*, *ermB*, *parC* and *folP* genes

Primer		Sequence (5'-3')	Tm ˚C	Product size (bp)	Ref.
meF	Forward	CAGGGTCATAAAGCCTAAATAG	60.0˚C	432	Rubio-López et al. (2012)
	Reverse	GAGGTAAGCTACATAAACTGTG	59.9˚C		
ermB	Forward	ATTGGAACAGGTAAAGGGC	60.1˚C	424	Sağıroğlu et al. (2011)
	Reverse	GAACATCTGTGGTATGGCG	60.1˚C		
parC	Forward	GGATTGAAACCCGTTCAGCG	59.9˚C	429	Rivera et al. (2005)
	Reverse	CTGGTAAAACGGTGGGTTCT	60.1˚C		
folP	Forward	GGGATCCAGGAGAGGACTATGAAGATT	60.0˚C	447	Swedberg et al. (1998)
	Reverse	AATGCTTTCCTCACATCAACTGACTCA	57.7˚C		

PCR amplification was carried out in a 25µL master mix (2X concentration), 2µL of each primer (10pmol/L) (Promega, USA), and 5µL of DNA extracted in a total volume of 50µl with sterile H_2O Diethyl pyrocarbonate (DEPC) treatment. The Veriti 96-well Thermal Cycler from Applied Biosystems was used to create the cycling conditions for gene detection (Puspanadan et al., 2013).

The PCR products were then placed onto a 1.5 percent agarose gel (vivantis, USA) and electrophoresed at 100V for around 30min in 1 X TBE buffer. The gels were then dyed with 2µL of 10mg/mL ethidium bromide (Sigma, USA). UVP-gel documentary system was used to photograph DNA bands visualised (UV Pdual-intensity transilluminator, model: TM-20) at wavelength 312nm (Hall & Beiko, 2018).

Sequencing of DNA gene fragment

Sequencing was done with "ABI 3730xl DNA sequencer" and Sequence Analysis Software v3.1. Basic Local Alignment Search Tool (BLAST) and BLAST nucleotide (BLASTN 2.2.13) tools were used to compare the sequences and homology of the four genes to the GenBank database (GATC Company, Germany) (Ibrahim et al., 2016).

Preparation of Ginger (Z. officinale)

The Experimental Farm of the Faculty of Pharmacy, Cairo University, Egypt, provided rhizomes of the *Z. officinale* plant. For 7 days, fresh plant samples were air-dried in a dark chamber at room temperature (28˚C). The herbs were powdered after being dried.

Z. officinale plant samples (500g) were hydro-distilled for 4h using a Clevenger-type apparatus, as indicated in the European Pharmacopoeia. The essential oil samples were kept at 4°C in the dark (Mahdavi et al., 2018).

Chemical characterization of essential oils

The essential oil was chemically characterized by gas chromatography–mass spectrometry (GC–MS) using a Agilent 7000 series Triple Quad Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS equipped with an Elite-5MS (5% diphenyl / 95% dimethyl poly siloxane)) fused a capillary column for GC-MS detection an electron ionization system with ionizing energy of 70ev was used.

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was turbo mass. Interpretation of mass spectrum GC/MS-MS was conducted using the database of National Institute of Standard and technology (NIST) (Akande, 2012).

Detection of minimal inhibitory and bactericidal conc. of Z. officinale (MIC and MBC)

Serial dilution of *Z. officinale* essential oil was done in Distilled water (1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100µg/mL) to study their effect on MDR *S. pyogenes,* which was subcultured overnight on Mueller-Hinton agar and to determine the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) (Hansen et al., 2017).

The experiment was done in triplicate, each tube contain 2mL Nutrient broth, 20µL bacterial suspension, and 50µL essential oil of each *Z. officinale* dilution. Positive control; tubes contain *Z. officinale* essential oil suspension only, while negative control; tubes contain bacterial suspension without *Z. officinale*. All tubes were incubated for 24h at 37°C before being measured at 600nm on a spectrophotometer (T80 UV/VIS Spectrometer, United Kingdom) (Mutlu-Ingok et al., 2021).

Results

Sixty isolates out of 120 (50.0%) were identified as *S. pyogenes*. 45 (75%) were detected from throat swaps samples and 15 (25%) from blood samples. These isolates were first identified morphologically, examined on blood agar plates for the presence of β-hemolytic colonies. The VITEK 2 system version 9.02 was used to analyze the biochemical features of these isolates, and positive results were determined as Pyrrolidonyl Arylamidase, Fructose, Galactose, Glucose, Lactose, Maltose, Salicin, Sucrose, and Trehalose fermenter. They also showed negativity for Catalase, Urease, Voges Proskauer, Adonitol, Arabinose, Arabitol, Dulcitol, Erythritol, Hippurate, Mannitol, Melibiose, Ribose, Sorbitol and Xylose tests.

Prevalence of *S. pyogenes* in clinical samples with co-relation to patient gender and age was detected. Males were more infected than females with a percentage of (63% and 37%) respectively and with age average between 20-60 years old (Fig. 1).

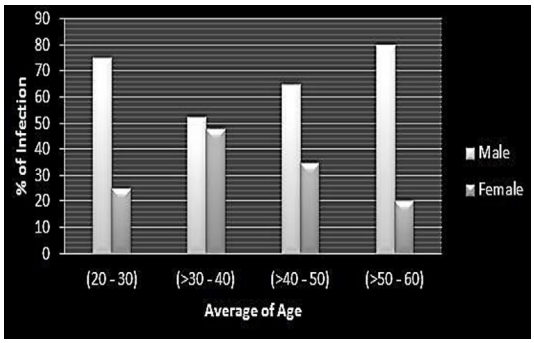

Fig. 1. Prevalence of *S. pyogenes* in clinical samples with co-relation to patient age

Sixty *S. pyogenes* samples were evaluated for antibiotic susceptibility using the disc diffusion plate method. Sixty percent of them were found to be multi-drug resistant strains (Fig. 2).

PCR were performed to determine the presence of *meF* (432bp), *ermB* (424bp), *parC* (429bp) and *folP* (447bp) genes responsible for multi-drug resistance in *S. pyogenes* strains. The PCR amplification products revealed that 19 strains out of 36 (52.8%) harbored the *meF* gene, 26 isolates (72.2%) harbored the *ermB* gene, 36 isolates (100.0%) harbored the *parC* gene, and 36 isolates (100.0%) harbored the *folP* gene. In contrast, nine isolates (25%) harbored the four genes (Figs. 3-6). Sequence alignment was done for each detected gene and compared to the World Wide Web's gene bank database, revealing the presence of several types of mutation (Table 2).

The antibacterial activity of different dilution of essential oil of *Zingiber officinale* against MDR *S. pyogenes* isolates harbored the four genes (*meF, ermB, parC,* and *folP*) was assessed as optical density to determine the MIC and MBC (Table 3). The table showed that the MBC was 20µg/mL, while the MIC was 10µg/mL.

The essential oil of *Zingiber officinale* was chemically characterized by gas chromatography-mass spectrometry (GC–MS) using the Agilent 7000 series Triple Quad Gas Chromatograph interfaced with a Mass Spectrometer. The chemical structure and concentrations of each component of the essential oil of *Zingiber officinale* were determined, as illustrated in Fig. 7.

Figure 7 illustrated the concentrations of chemical compounds detected in *Zingiber officinale* essential oil. 7-Methoxymethyl-2,7-dimethylcyclohepta-1,2,5-triene compound was detected with a percentage of 1.79%, Bicyclo(4.2.0)oct-1-ene, 7-exo-ethenyl compound with percentage of 5.59%, trans-beta.-Ocimene compound with percentage of 2.25%, Pentadecanoic acid, 14-methyl-,methyl ester compound with percentage of 1.61%, Nonanoic acid compound with percentage of 4.61%, Methyl 12,13-tetradecadienoate compound with percentage of 16.9%, 17-Octadecynoic acid compound with percentage of 48.27%, Cyclohexanone, 2-(2-nitro-2-propenyl) compound with percentage of 2.38%, 13-Tetradece-11-yn-1-ol compound with percentage of 4.0%, 17-Octadecynoic acid compound with percentage of 4.64%, 13-Tetradece-11-yn-1-ol compound with percentage of 0.96% and 9,12-Octadecadienoyl chloride compound with percentage of 6.53%.

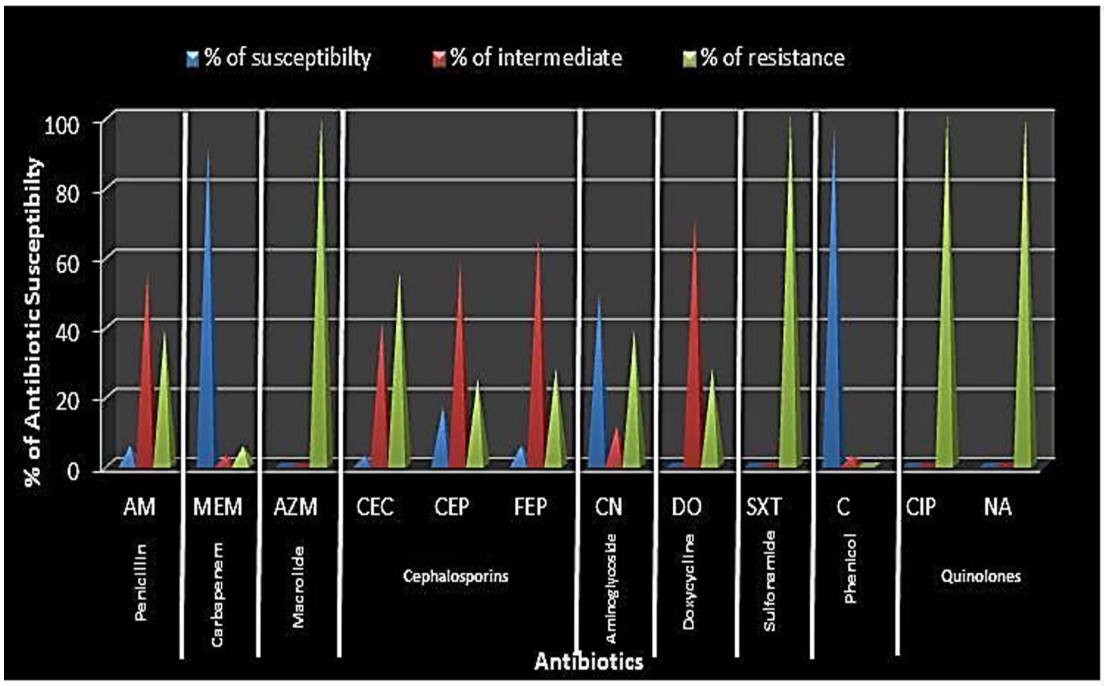

Fig. 2. Percentage of susceptible (S), intermediate (I) and resistance (R) for multi-drug resistant *S. pyogenes.* AM (Ampicillin), MEM (Meropenem), AZM (Azithromycin), CEC (Cefaclor), CEP (Cefoperazone), FEP (Cefepime), CN (Gentamycin), DO (Doxycycline), SXT (Trimethoprim/Sulphamathoxazole), C (Chloramphenicol), CIP (Ciprofloxacin) and NA (Nalidixic acid)

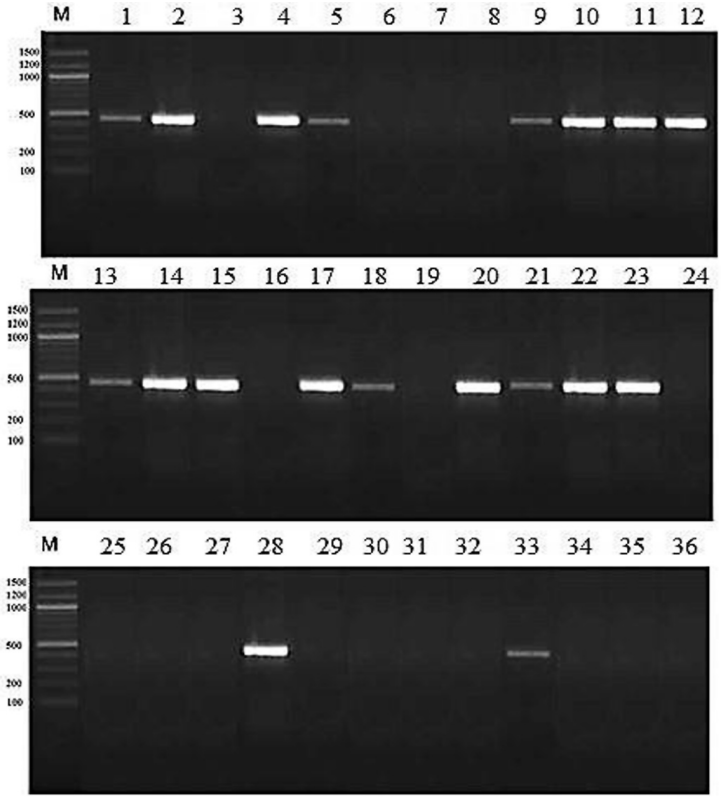

Fig. 3. Amplified 432bp DNA fragment *meF* gene of MDR *S. pyogenes* [M as Marker & 1, 2, 3, ….to 36 as no. of sample]

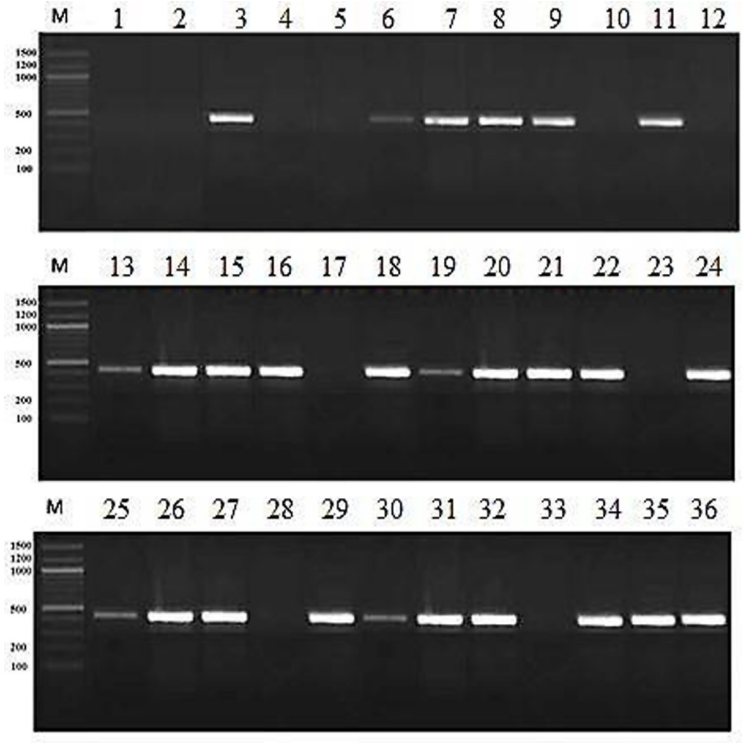

Fig. 4. Amplified 424 bp DNA fragment *ermB* gene of MDR *S. pyogenes* [M as Marker & 1, 2, 3, ….to 36 as no. of sample]

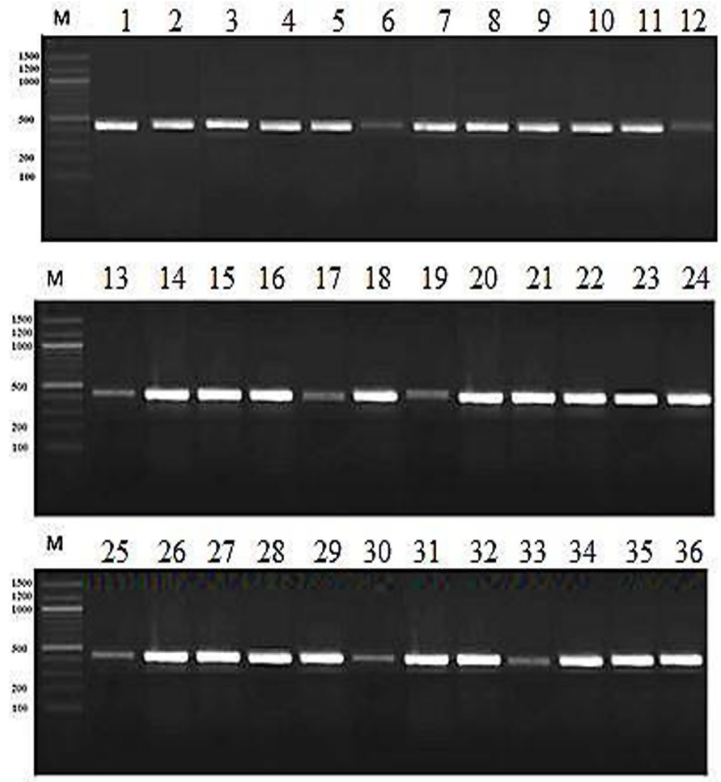

Fig. 5. Amplified 429 bp DNA fragment *parC* gene of MDR *S. pyogenes* [M as Marker & 1, 2, 3, ….to 36 as no. of sample]

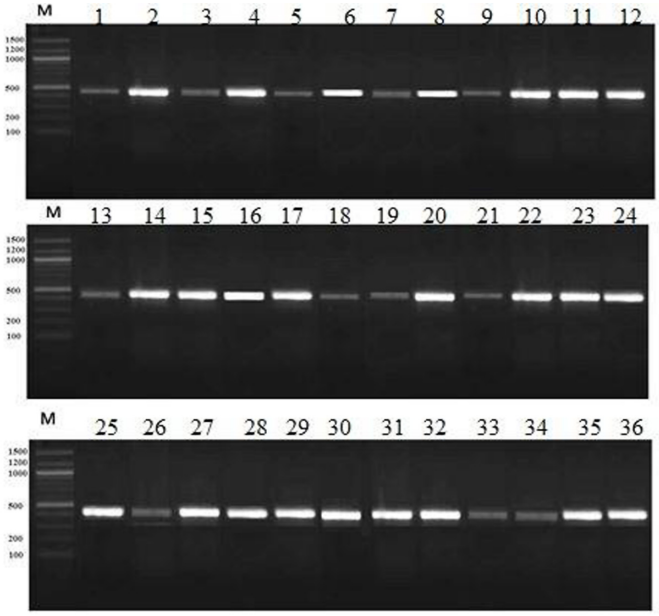

Fig. 6. Amplified 447 bp DNA fragment *folP* gene of MDR *S. pyogenes* [M as Marker & 1, 2, 3, ….to 36 as no. of sample]

TABLE 2. Mutation types detected in complex region of *meF, ermB, parC and folP* genes of MDR *S. pyogenes* compared to GenBank data base

Multi-drug resistant genes	Types of mutations		
	Substitution (Inversion)	Substitution (Transversion)	Deletion
meF gene	Q148: G-A; Q388: A-G	--	--
ermB gene	Q152: T-C	Q100: T-G	--
parC gene	Q77: G-A; Q104: T-C;; Q354: T-C Q406: G-A	Q40: T-G; Q163: A-T; Q165: A-C	--
folP gene	Q438: A-G	--	Q436: -C Q437: -T

Note/ Q: Query position, A: Adenine, G: Guanine, T: Thymine and C: Cytosine.

TABLE 3. Antibacterial activity of different concentrations of *Ginger (Zingiber officinale)* on MDR *S. pyogenes* strain

Concentrations of *Ginger (Zingiber officinale)* (µg/mL)	Measuring on spectrophotometer at 600nm (optical density)
	Antibacterial Activity of *Ginger (Zingiber officinale)* on MDR *S. pyogenes* have four genes (nm)
1	1.180
10	1.176 (MIC)
20	1.050 (MBC)
30	1.201
40	1.208
50	1.360
60	1.380
70	1.400
80	1.408
90	1.440
100	1.460

Note: Positive control (treated *S. pyogenes*) 0.980nm
Negative control for (*S. pyogenes* without treatment) 1.890nm

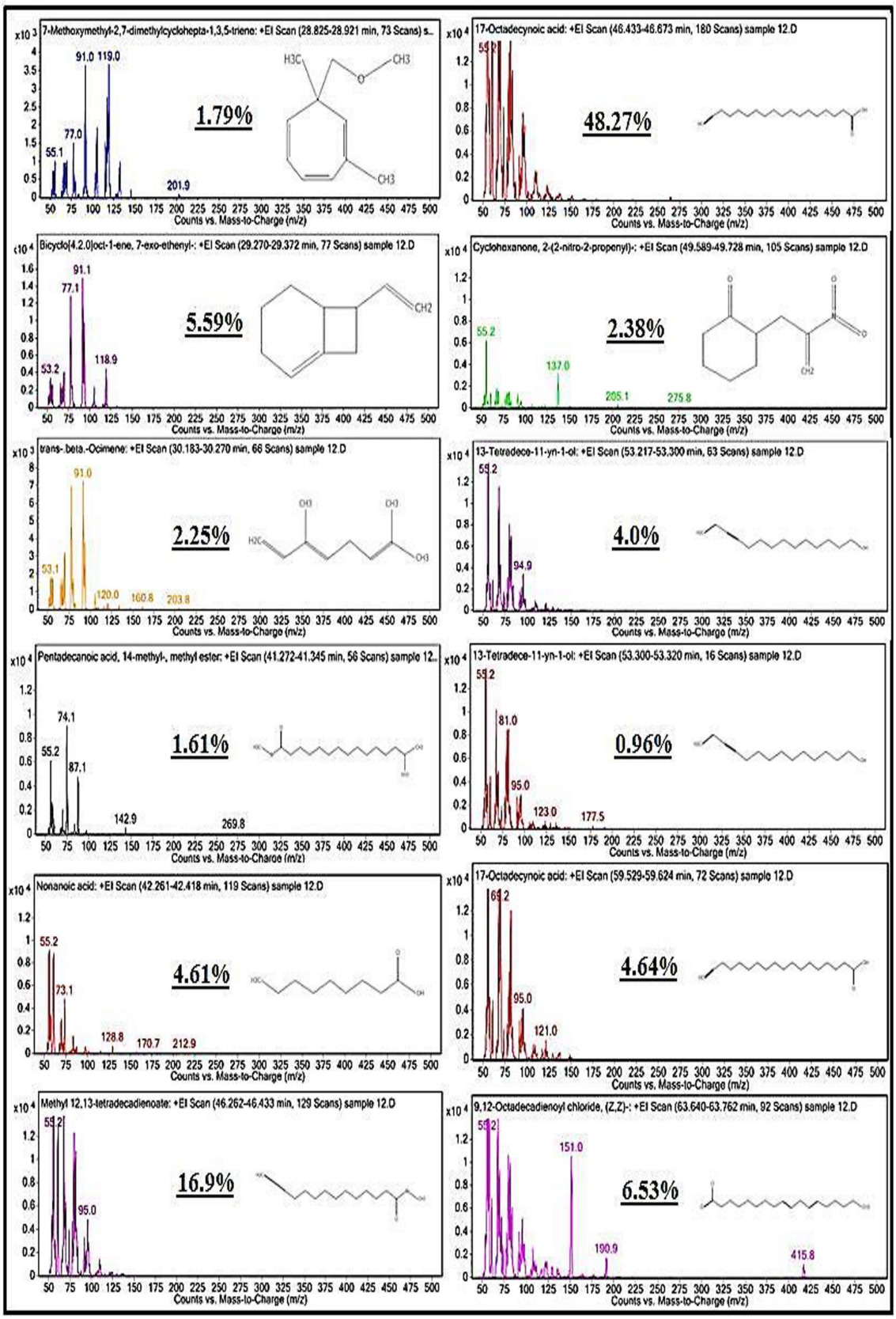

Fig. 7. Diagrams showing GC-MS for chemical composition, structure, and concentrations of *Zingiber officinale* essential oil

Discussion:

In the current study, sixty isolates out of 120 (50.0%) clinical specimens were identified as *S. pyogenes*. 45% were detected from throat swaps samples, while 15% were from blood samples. Males were more infected with a percentage of 63% than females with a percentage of 37% with age average between 20-60 years old.

The high prevalence rate of GAS found in this study could be due to a variety of causes, including student congestion in class, hunger, and a lack of awareness of personal hygiene and also to our traditions that males are more exposed to environmental conditions than females who often stay at home.

AL-Taei et al. conducted a similar investigation (2016). They found that *S. pyogenes* grew 3.07 percent from 260 samples, with blood accounting for 75.8% and throat swab accounting for 24.2 percent. Throat swabs were collected from children admitted to the Child Protection Hospital, the Baghdad Teaching Hospital, the Specialist Surgery Hospital, and the Central Child Hospital between March and July 2014.

Similarity, to this result AL-Taei et al., (2016) illustrated that the frequent pathogens isolated from the patients admitted to Baghdad, were higher in males (65.0%) than females (35.0%).

This result was in a contradiction with Othman et al. (2019), who discovered a statistically significant greater frequency of GAS in females (66.15 percent) than in males (38.10 percent). Females were exposed to *S. pyogenes* infection at a higher rate than males, according to similar findings (Vijaya et al., 2013; Nayiga et al., 2017; Anja et al., 2019).

Only 60% (36/60) of the sixty *S. pyogenes* isolates recovered from the gathered clinical specimens were confirmed to be multi-drug resistant strains, according to this study.

The existence of the *meF, ermB, parC*, and *folP* genes responsible for multi-drug resistance in *S. pyogenes* strains was determined by PCR in this work. The PCR product revealed that 19 strains out of 36 (52.8%) carried the *meF* gene, 26 isolates (72.2%) carried the *ermB* gene, 36 isolates (100.0%) carried the *parC* gene, and 36 isolates (100.0%) carried the *folP* gene, whereas nine isolates (25%) carried both four genes.

The other finding detected Camara et al. (2013), who found that isolated *S. pyogenes* were completely (100%) resistant to tetracycline. According to Anja et al. (2019), tetracycline resistance was found in 42.9 percent of *S. pyogenes* isolates. Their multidrug resistance (MDR) character has been linked to serious healthcare difficulties in earlier investigations. Tetracycline resistance has been found in several *Streptococcus pyogenes* strains (Rashid, 2006).

Multidrug-resistant *S. pyogenes* was shown to be highly sensitive to chloramphenicol (97.22%) and Meropenem (91.66%) in this investigation.

Bley et al., (2011) conducted a study on the amplification of the *mef* and *ermB* genes and discovered that 31.0 percent of isolates had the *mef* gene present, while 34.5 percent had the *ermB* gene present.

Only 26.1 %t of *S. pyogenes* isolates expressed the *mef* gene in a previous investigation that looked for macrolide resistance genes. The *ermB* gene was found to be a determinant in the majority of erythromycin resistant isolates (65.2%) (Katosova et al., 2016).

Sequence alignment of the *meF, ermB, parC*, and *folP* genes for the Egyptian *S. pyogenes* strain was done on the gene bank database on the World Wide Web and revealed the presence of several alterations, according to the current study. Some mutations in the four genes ranged between inversion mutation in *meF* gene, substitution mutation in *ermB* and *parC* genes and deletion and inversion mutations in *folP* gene.

The rhizome of *Z. officinale* contains 1–4% essential oil and oleoresin, although sesquiterpene (53.57%) is the main ingredient (Nazish et al., 2016). Several molecules have been found in essential oil, with a total of 28 compounds estimated. Eudesmol (8.19%), -terpinene (7.88%), -curcumene (7.28%), zingiberene (6.06%), and alloaromadendrene (6.06%) were the most abundant (6.56 percent), α-pinene (5.76%), δ-cadinene (3.84%), elemol (3.39%), farnesal (3.45%), E-β-farnesene (3.57%), neril acetate (2.8%) and β-myrcene (2.94%) (López et al., 2017).

The present study indicated that 17-Octadecynoic acid was the most common compound with a percentage of 48.27%. 17-Octadecynoic acid, as structural characteristics that impart antibacterial activity to C_{18} alkynoic fatty acids (aFAs) to form micelles can be linked to their decreased activity of Gram-positive bacteria, its effect on protein of bacteria and inhibition of the bacterial fatty acid synthesis (David et al., 2020).

Conclusion

The present study showed that *Zingiber officinale* essential oil was found to be highly effective on MDR *S. pyogenes* that contain (*meF, ermB, parC,* and *folP*) resistant genes. The minimal inhibitory concentration (MIC) was 10% and the minimal bactericidal concentration (MBC) was 20%. 17-Octadecynoic acid was the most prevalent chemical present in this oil and many researches detected its antibacterial effect.

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الخصائص الجزيئية للمكورات العقدية المقيحة المتعددة المقاومة للادوية والقضاء عليها باستخدام مستخلص زيت الزنجبيل أوفيسينال كعامل طبيعي مضاد للميكروبات

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تعد المكورات العقدية المقيحة المتعددة المقاومة للادوية من المسببات للامراض المختلفة. حيث انها تنتج انزيمات جديدة تستطيع ان تكسر المضادات الحيوية اكثر من معظم البكتريا الاخرى. هذه الدراسة تهدف إلى اكتشاف و تعريف المكورات العقدية المقيحة بواسطة طريقة التنميط المصلي خاصة المكورات العقدية المقيحة المتعددة المقاومة للادوية مع اكتشاف وتسلسل جينات *meF* و *ermB* المسؤولة عن مقاومة للمضادات الحيوية (الماكرولايد)، وجين *parC* المسؤول عن مقاومة المضاد الحيوى (الكينيلون) والجين *folP* المسؤول عن مقاومة المضاد الحيوى (السلفونااميد). واستخدام مستخلص زيت الزنجبيل أوفيسينال كعلاج طبيعى للمكورات العقدية المقيحة المتعددة المقاومة للادوية. تم اكتشاف المكورات العقدية المقيحة 60/120 عينات من الحلق والدم بنسبة 50.0٪. 36/60 عينة التى اظهرت المقاومة العديد من الادوية. كان الرجال أكثر عرضة للإصابة من النساء. النتائج تشير إلى 52.8٪ من المكورات العقدية المقيحة تحتوي على جين *meF*، 72.2٪ تحتوي على جين *ermB* ، 100.0٪ تحتوي على جين *parC* و 100.0٪ تحتوي على جين *folP* بينما 25٪ منها تحتوي على الجينات الأربعة. أظهرت المحاذاة التسلسلية لجينات meF و ermB و parC و folP بعض الطفرات في الجينات الأربعة التي تراوحت بين طفرة الانعكاس في جين meF، وطفرة الاستبدال في جيناتermB و parC وطفرات الحذف والاستبدال في جين folP. تم تحليل مستخلص زيت الزنجبيل أوفيسينال بواسطة GC-MSوالذي أوضح أن حمض Octadecynoic- 17 كان المكون الأكثر شيوعًا بنسبة 48.27 ٪. وجد أن 20 ميكروغرام / مل هو أقل تركيز مبيد لبكتريا المكورات العقدية المقيحة المتعددة المقاومة للمضادات الحيوية والتى تحتوى الاربع جينات.