ANTIBACTERIAL ACTIVITY OF METHYLGLYOXAL AGAINST MULTI-DRUG RESISTANT SALMONELLA TYPHI

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ABSTRACT

Objective: To evaluate the antibacterial activity of MGO against MDR Salmonella typhi isolated from blood culture specimens and compare this activity against non-MDR *S. typhi* and with other gram negative rods.
Study Design: Experimental study.
Place and Duration of Study: Department of Microbiology, University of Health Sciences Lahore, from Jul 2011 to Jun 2012.
Material and Methods: A total of 157 isolates of *S. typhi* were collected from different hospitals of Lahore and kept stored at -80°C. Morphological, biochemical and serological identification and antibiotic susceptibility testing of the isolates was carried out as per CLSI 2011 guidelines. Agar dilution method was used for the determination of MICs of MGO, using a multi-point inoculator. The data was compiled and results were determined using SPSS version 17.
Results: Ninety-seven out of 157 isolates (61.8%) were MDR *S. Typhi*, while 60 (38.2%) were non-MDR S. Typhi. MIC90 of MGO against MDR *S. Typhi* isolates was (0.20 mg/mL; 2.8 mM), against non-MDR *S. Typhi* and Gram negative rods each, it was (0.21 mg/mL; 3.0 mM). When MICs of MGO against MDR *S. Typhi* group were compared to those of non-MDR *S. Typhi* group, the *p*-value was 0.827 ($p>0.05$; statistically insignificant). Whereas, the *p*-value of MICs of MGO against MDR S. Typhi group was 0.023 ($p<0.05$; statistically significant) when compared to gram negative rods group.
Conclusion: MGO has good antibacterial activity against MDR and non-MDR S. Typhi, and other genera of Gram negative rods.

Keywords: MDR Salmonella Typhi, Methylglyoxal (MGO), Typhoid

INTRODUCTION

Typhoid remains a major cause of morbidity and mortality in developing countries. There were estimated 22 million new cases globally with over 600,000 deaths as mentioned in WHO 2011 report[1]. Majority of cases (93%) are reported from Asia[2]. Emergence of multidrug resistance and reduced ciprofloxacin susceptibility in Salmonella Typhi rendered conventional drugs ampicillin, chloramphenicol and co-trimoxazole ineffective or suboptimal for treatment[3]. The negative development was in the form of an increase in multi-drug resistant (MDR) isolates throughout the world, which is a simultaneous resistance to first-line conventional anti-typhoid drugs; ampicillin, co-trimoxazole and chloramphenicol[4]. We face the very real prospect that untreatable typhoid fever will emerge sooner or later[5]. Consequently, efforts have to be made to evaluate new antibacterial agents as the anti-typhoid agents.

Honey on the basis of its wide antibacterial activity is naturally becoming a choice, therefore. The antibacterial properties of honey are mainly attributed to its 'bee origin' factors such as acidic pH, high osmolarity and release of hydrogen peroxide by glucose oxidase and 'plant origin' non-peroxide factors like methylglyoxal (MGO)[5]. The speculated concentration of MGO in manuka honey (*Leptospermum scoparium*), for example, is up to 100-fold higher than in other flora of honeys[6]. MGO, a dicarbonyl compound, acts by formation of advanced glycation end products (AGEs) and glutathione adducts in the cytoplasm

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of cells, including bacteria. It is found to be a highly reactive precursor[7]. It reacts with thiol groups of proteins causing inhibition of some enzyme activities thus rendering the proteins non-functional. Previously, MGO in manuka honey was labelled as "Unique Manuka Factor" (UMF)[8].

Keeping in view of the vast unexplored potential for application of MGO within the clinical environment, it is essential that research should continue beyond the topical application of honey and its derivative MGO, in fact, to systemic infections particularly where conventional therapy is tending to fail. The objectives of the study were to evaluate the antibacterial activity of MGO against MDR S. Typhi isolated from clinical cases and to compare the same against that of non-MDR *S. Typhi*, and various genera of Gram negative rods.

MATERIAL AND METHODS

It was an experimental study conducted at the Department of Microbiology, University of Health Sciences (UHS) Lahore-Pakistan from July 2011 to June 2012, in collaboration with the Department of Microbiology, Combined Military Hospital Lahore. All microbiological procedures were carried as per CLSI 2011 guidelines.

Recently isolated 157 *S. Typhi* from clinical blood culture specimens were collected from various hospitals of Lahore and were stored at -80°C. Sample size was calculated with WHO sample size calculator with Confidence Interval of 95% and anticipated population proportion of 98%. The isolates were thawed and identified on the basis of their colony morphology and morphology with Gram stain, after sub-culturing on recommended culture media. Motility was observed and the isolates were tested for catalase and oxidase enzymes production. Biochemical identification was done by using API-20 E (bio-Merieux, France) strips. Serological identification was performed by polyvalent and group specific Salmonella O, H and Vi antisera (BD Difco, USA), as per standard serological protocol.

Antibiotic susceptibility testing of the isolates was performed by Kirby-Bauer disc diffusion method using Meuller-Hinton agar (MHA) (Oxoid Ltd, UK). Anti-typhoid antibiotic discs (Oxoid Ltd, Basingstoke, UK) were applied with their respective disc contents; ampicillin (10µg), co-trimoxazole (1.25/23.75µg), chloramphenicol (30µg), nalidixic acid (30µg), ciprofloxacin (5µg), ceftriaxone (30µg), aztreonam (15µg) and imipenem (10µg). The plates were incubated at 35°C aerobically for 18-24 hours. Zones of inhibition were noted in millimeters (mm). MDR *S. Typhi* group comprised those isolates which were simultaneously resistant to ampicillin, co-trimoxazole and chloramphenicol, remaining were grouped as non-MDR S. Typhi.

MGO was purchased from its authorized supplier MP Pharma, 29525 Fountain Parkway Solon, OH 44139, USA. Its sterility and expiry were confirmed before use. MGO was tested for its MICs by agar dilution method against 157 clinical isolates of *S. Typhi* (MDR and non-MDR), 33 indigenous clinical Gram negative rods (GNRs) and 6 ATCC (American Type Culture Collection) control stains. A multi-point inoculator (Mast Diagnostics, England) was used for inoculating multiple isolates simultaneously. Serial dilutions of MGO, to be incorporated into MHA, were made from 0.08 mg/mL to 0.24 mg/mL, based on its molecular weight 76.02. The plates were incubated at 35°C for 18-24 hours aerobically.

As this was an ever new study and no standard/control strains were available, therefore indigenous clinical GNRs isolated from blood culture specimens and ATCC control strains were used to monitor the study and validate the experimental work[9]. GNR isolates (n=33) were *Escherichia coli* (n=18), *Klebsiella pneumoniae* (n=6), *Enterobacter cloacae* (n=2), *Klebsiella oxytoca* (n=2), *Citrobacter braakii* (n=2), *Enterobacter agglomerans* (n=2) and *Enterobacter aerogenes* (n=1). The ATCC control strains (n=6) used were *Staphylococcus aureus* ATCC 25923 (n=1), *Escherichia coli* ATCC 25922 (n=1), *Klebsiella pneumoniae* ATCC 700721 (n=1), *Salmonella S. Typhimurium* ATCC 39183

(n=1), *Acinetobacter baumanii* ATCC 19606 (n=1) and *Klebsiella oxytoca* ATCC 700324 (n=1). Additionally, internal quality control comprised three MHA plates. First plate contained MHA with incorporated MGO, second contained MHA with MGO but inoculated with sterile pins of the multi-inoculator, and the third plate contained MHA without MGO but inoculated with pins of the multi-inoculator dipped in test and control isolates.

MIC was defined as the lowest concentration of MGO at which there was no visible bacterial growth on agar surface when observed with the unaided eye against a dark background. The data was interpreted according to defined criteria of MIC range, MIC50 and MIC90. The data was further sorted out by the computer software Statistical Package for Social Sciences (SPSS) v.17.0.1. Independent sample t-test was applied to see the difference in antibacterial activity of MGO among all groups of isolates independently. For all statistical tests, results were considered to be significant at *p*-value<0.05.

RESULTS

A total of 157 *S. Typhi* isolates were tested against a panel of antibiotics as per CLSI recommendations. Ninety-seven (61.8%) isolates were MDR S. Typhi, remaining 60 (38.2%) were non-MDR S. Typhi. The first two internal control plates did not show any growth, while all organisms showed growth on the third control plate. All ATCC control strains also showed growth. The range of MICs of MGO against both MDR and non-MDR *S. Typhi* groups was same (0.14 to 0.24 mg/mL; 2.0 to 3.4 mM), while it was slightly narrower (0.16 to 0.22 mg/mL; 2.3 to 3.1 mM) against Gram negative rods group. The MIC90 of MGO against non-MDR *S. Typhi* group was (0.20 mg/mL; 2.8 mM), while it was slightly higher against both non-MDR S. Typhi and Gram negative rods groups (0.21 mg/mL; 3.0 mM) (tables-I, II, III).

Table-I: MICs of MGO against MDR S. Typhi (n=97).

	MICs of MGO		
	Range	**MIC50**	**MIC90**
In mg/mL	0.14 to 0.24	0.19	0.20
In mM	2.0 to 3.4	2.7	2.8

Table-II: MICs of MGO against Non-MDR S.Typhi (n=60).

	MICs of MGO		
	Range	**MIC50**	**MIC90**
In mg/mL	0.14 to 0.24	0.19	0.21
In mM	2.0 to 3.4	2.7	3.0

Table-III: MICs of MGO against Clinical GNR Isolates (n=33).

	MICs of MGO		
	Range	**MIC50**	**MIC90**
In mg/mL	0.16 to 0.22	0.20	0.21
In mM	2.3 to 3.1	2.8	3.0

The Mean ± SD of both MDR S. Typhi and non-MDR S. Typhi groups were 0.05 mg/mL/ 0.7 mM, whereas, the Mean ± SD of GNR group was 0.03 mg/mL/ 0.4 mM. Independent sample t-test showed that the *p*-value of MICs of MGO against MDR *S. Typhi* group when compared to non-MDR *S. Typhi* group was 0.827 ($p>0.05$; statistically insignificant), Mean ± SD 0.05 ± 0.03. The *p*-value of MICs of MGO against MDR S. Typhi group when compared to Gram negative rods group was 0.023 ($p<0.05$; statistically significant), Mean ± SD 0.04 ± 0.02.

DISCUSSION

In Pakistan, researchers have reported a persistently increased prevalence of MDR *S. Typhi* over the years, 53.8% (2001)[10], 64.2%

(2006)[11], 48.5% (2008)[12]. This study with 61.8% MDR *S. Typhi* conforms to the ongoing trend in Pakistan. Major contributing factors in the development of multi-drug resistance have been identified as injudicious use, inappropriate prescribing practices and intrinsic plasmid mediated factors. The presence of other virulence genes on R-plasmid may result in increased virulence of MDR strains. Severe complicated illnesses and high mortality has been reported due to MDR *S. Typhi*[14].

To-date, to the best of our knowledge and the literature cited, MGO has not yet been tested for its antibacterial activity against MDR S. Typhi. In this study, the MIC90 of MGO against MDR *S. Typhi* isolates (0.20 mg/mL; 2.8 mM), non-MDR *S. Typhi* (0.21 mg/mL; 3.0 mM), clinical GNR isolates having *E. coli* strains (n=18) (0.21 mg/mL; 3.0 mM) and control strains of *E. coli* ATCC 25922 (0.20 mg/mL; 2.8 mM) and *S. aureus* ATCC 25923 (0.15 mg/mL; 2.2 mM) are relatively but proportionately high when compared to a study on MGO, where the MIC against *E. coli* and *S. aureus* was comparatively low (0.08 mg/mL; 1.1 mM)[6]. This comparative difference can be attributed to the method used for MIC determination. Agar well diffusion method used by Mavric et al, lacks sensitivity and proper standardization as compared to the agar diffusion method, affecting the outcome of assay[23].

A group of researchers in 2010 inducted MGO in a hydrogel for its tropical antibacterial action. Their MICs against *S. aureus* and methicillin-resistant *Staphylococcus epidermis* (MRSE) were low (0.07 mg/mL; 1.05 mM)[24]. The MIC90 values (0.20 mg/mL; 2.8 mM) in this study were comparable with a variation towards higher side, but Fidaleo et al, had used broth dilution assay for MIC determination. Moreover, the isolates in this study belonged to different genera and clinical specimen sources. However, a control isolate in this study *S. aureus* ATCC 25923 showed comparable MIC (0.15 mg/mL; 2.2 mM). In 2011, another group of researchers reported an effective concentration range of MGO against MRSA (0.08 to 0.3 mg/mL; 1.1 to 4.3 mM), and against *P. Aeruginosa* (0.15 to 1.2 mg/mL; 2.1 to 16.4 mM)[25]. The range of MIC of MGO against MDR S. Typhi in this study (0.16 to 0.22 mg/mL; 2.3 to 3.1 mM), however is comparable.

As a future prospective antibacterial agent, further studies are required to be carried out on MGO in order to establish its safety profile in the human body. This would provide headway for it to be used against other systemic bacterial infections as well.

CONCLUSION

It was concluded that MGO has good antibacterial activity against MDR *S. Typhi*, non-MDR *S. Typhi* and various other genera of Gram negative rods.

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CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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