In vitro and *in vivo* activity of Manuka honey against the NDM-1 producing *Klebsiella pneumoniae* ST11

Qamar MU*,[1,2], Saleem S[1], Toleman MA[3], Saqalein M[2], Waseem M[3], Nisar MA[2], Khurshid M[2,4], Taj Z[2] & Jahan S**,[5]

[1]Department of Microbiology, University of Health Sciences, Lahore, Pakistan

[2] Department of Microbiology, Faculty of Life Sciences, Government College University Faisalabad, Pakistan

[3]School of Medicine & Infectious Disease, University Hospital of Wales, Heath Park, Cardiff University, Cardiff, United Kingdom

[4]College of Allied Health Professionals, Directorate of Medical Sciences, Government College University, Faisalabad, Pakistan

[5]Department of Immunology, University of Health Sciences, Lahore, Pakistan

*Author for correspondence: Tel: +923013054705; musmanqamar@gcuf.edu.pk , usman9785@gmail.com

** Author for correspondence: Tel.: +92 30 0508 1072; shahjahan@uhs.edu.pk

Abstract

Aim: To determine the therapeutic potential of Manuka honey against NDM-1 producing *K. pneumoniae* ST11 *in vitro* and *in vivo*. **Materials and Methods:** Carbapenamases and MBLs producing *K. pneumoniae* ST11 isolated from blood culture was confirmed by Vitek 2, MALDI-TOF, MLST and MIC (μg/mL) was performed by Vitek 2 system. Genetic analysis of bla_{NDM-1} was done by PCR, PFGE and DNA hybridization. *In vitro* and *in vivo* efficacy of Manuka honey was performed by microbroth dilution assay and *BALB/c* mice respectively. **Results:** *K. penumoniae* ST11 displayed resistance to commonly used antibiotics. bla_{NDM-1} was located on 150kb of plasmids. MIC and MBC of Manuka honey was 30% (v/v) and substantial reduction of bacterial mean log value (>1log) was observed in mouse model. Histological analysis of mice liver and kidneys demonstrated mild to moderate inflammation. **Conclusion:** Manuka honey can be used as alternate therapeutic approach for the management of NDM producing pathogens.

Key Words: *Klebsiella pneumoniae*, MLST, NDM, Manuka honey, BALB/c

Introduction

New Delhi Metallo-β-lactamase (NDM) is an emerging type of carbapenamase belongs to the B1 super family of metallo-β-lactamases (MBLs) are rapidly spreading globally [1]. NDM was first identified from *Escherichia coli* and *Klebsiella pneumoniae* isolated from a Swedish patient who had been hospitalized and sought medical care in New Delhi, India [2]. NDM producing pathogens confer resistance against a wide range of antibiotics including aminoglycosides, quinolones, monobactam and most notably β-lactam drugs including carbapenems; considering last resort to treat extensively drug resistant (XDR) pathogens [3]. NDM producing bacteria are responsible for high rate of mortality in our clinical settings. Previously, we reported that 4/9 pediatrics who had been hospitalized in Islamabad, Pakistan died due to NDM producing bacteria [4]. Recently, another study revealed that 57% and 60% adult and neonatal patients died respectively in Karachi, Pakistan and the chief pathogen was NDM-1 positive *K. penumoniae* [5]. NDM producing pathogens confer resistance against a wide range of antibiotics including aminoglycosides, quinolones, monobactam and most notably β-lactam drugs including carbapenems; considering last resort to treat extensively drug resistant (XDR) pathogens and no or few treatment options available [3]. Therefore, therapeutic management of NDM producing bacteria is becoming a big challenge worldwide. Over the century, there is a huge production and development of synthetic drugs to improve the health care problems however, many countries still rely on their traditional medicine like honey as a primary care [6].

Manuka honey is one the widely used medicinal honey for wound dressing worldwide. Its antibacterial activity is mainly due to the presence of methylglyoxal (MGO) compound apart from acidic pH, high osmotic pressure, immune stimulant and the presence of trace elements [7]. Hence, Manuka honey is promising for the extensive cell lysis of bacteria and it is hard to believe that bacteria produce resistance against its multi-directional effects because it contains a combination of different compounds that may act synergistically to overcome antibacterial resistance. Previously, there are several *in vitro* studies have been

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reported in different regions on the antibacterial nature of Manuka honey against multi-drug resistant (MDR) pathogens [8-10]. In our previous study, we produced septicemia in *BALB/c* model following the methicillin resistant *Staphylococcus aureus* (MRSA) infection and treat them with Manuka honey intravenously (data not published). All the mice were recuperated, and no mice mortality were noticed [11]. As per our knowledge, there is no data available on the efficacy of Manuka honey against the NDM producing pathogens in mouse model.

Therefore, this study aimed to determine *in vitro* and *in vivo* activity of Manuka honey against the NDM-1 producing *K. pneumoniae* sequence type (ST) 11.

Materials and Methods

Identification and confirmation of bacterial strain

Klebsiella pneumoniae isolated from blood culture was collected from a tertiary care hospital Lahore. Bacterial isolate was sub-cultured on blood, MacConkey and UTI CHROMagar™ and plates were incubated at 37°C overnight. Preliminary identification was done based on colony morphology and cultural characteristics. Biochemical identification was done by utilizing 64 different biochemical tests on VITEK® 2 system (bioMérieux, France) using GN ID cards and peptide base bacterial confirmation was done by matrix assisted lase desorption ionization time of flight (MALDI-TOF) (Bruker Daltonics, Germany).

Sequencing typing (ST) of *K. pneumoniae*

Multilocus sequence typing (MLST) of *K. pneumoniae* was done by amplifying seven housekeeping genes including, *rpoB* (beta-subunit of RNA polymerase), *gapA* (glyceraldehyde-3-phosphate dehydrogenase), *mdh* (malate dehydrogenase), *pgi* (phosphoglucose isomerase), *phoE* (phosphorene E), *infB* (translation ignition factor 2), *tonB* (periplasmic energy transducer) by multiplex PCR as describe earlier [12]. PCR products were visualized on ethidium bromide stained agarose gel and product sizes were compared with ladder. Amplicons were extracted from gel using commercially available QIAquick Gel

extraction kit (QIAGEN, Germany) and sequenced by Eurofins Scientific Company, UK. After sequencing, data of seven genes were analyzed using Institute Pasteur MLST *K. penumoniae* database [13].

Antimicrobial susceptibility testing

MIC (μg/mL) of the *K. penumoniae* was performed using AST-XN 05 cards in VITEK$^®$2 compact instrument (bioMerieux, France) against 18 different antibiotics including Ampicillin/Sulbactam, Ticarcillin/Clavulanic Acid, Piperacillin, Cefuroxime, Cefuroxime axetil, Cefixime, Ceftriaxone, Cefepime, Aztreonam, Meropenem, Levofloxacin, Moxifloxacin, Minocycline, Tetracycline, Tigecycline, Chloramphenicol, Colistin and Trimethoprim. Results were interpreted as per Clinical Laboratory Standard Institute (CLSI) 2015 guidelines [14]. MIC (μg/mL) is the lowest concentration of an antibiotic that inhibits the growth of bacteria.

Phenotypic detection of carbapenamases

Carbapenamase detection was done using Modified Hodge's test (MHT) as per CLSI 2015 guidelines. Briefly, 0.5McFarland *E. coli* (ATCC 25922) lawned on Mueller Hinton agar (MHA) plate. A meropenem (10μg) disc was placed centrally and test organism, positive and negative control were streaked from edge of the disc to the edge of the MHA plate. Carbapenamases positive bacteria permit the meropenem sensitive *E. coli* (ATCC 25922) to grow against the meropenem disc which lead to a cloverleaf like indentation [14].

Phenotypic detection of Metallo-β-lactamases (MBLs)

MBLs detection was done by double disc synergy method. In short, MBLs belong to the class B of β-lactamases which require Zn^{+2} as a cofactor for their enzymatic activity. Their functional activity can be lost by treating them with a chelating agent such as ethylene diamine tetra acetic acid (EDTA). Briefly, 0.5McFraland bacterial suspension was lawned on the MHA plate. Two meropenem (10μg) and two ertapenem (10μg) discs were placed and 0.5M EDTA solution was added on one meropenem and ertapenem disc. Bacteria was MBLs

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producer if the EDTA discs showed >5mm zone of inhibition as compare to non EDTA discs [15].

Molecular identification of *bla*_{NDM-1}

Bacterial DNA extracted by commercially available bacterial genomic DNA kit TIANamp (China). *bla*_{NDM} was identified using specific primers: NDM-F 5′-ATGGAATTGCCCAATATTATGCAC-3′ and NDM-R 5′-TCAGCGCAGCTTGTCGGC-3′ using following conditions; initial denaturation: 95°C for 1 min, secondary denaturation: 95°C for 45 sec, annealing: 58°C for 45 sec, primary extension: 72°C for 1 min and final extension: 72°C for 5min and ∞ at 4°C. Amplicon was separated on ethidium bromide stained agarose gel using horizontal electrophoresis and product size was compared with DNA ladder.

Sequencing

Furthermore, *bla*_{NDM} was re-amplified by employing another set of primers (NDMV-F 5′-TGGCTTTTGAAACTGTCGCACC-3′ and NDMV-R 5′-CTGTCACATCGAAATCGCGCGA-3′). These primers were designed upstream and downstream of the gene to ensure the entire gene was sequenced. DNA sequence was analyzed by Geneious software [16] and aligned sequence was submitted to GenBank using BankIt software [17] for accession number allotment.

Plasmid characterization

Diversity of plasmids and *bla*_{NDM-1} location was done by S1 nuclease pulse field gel electrophoresis (PFGE) and DNA hybridization respectively as described previously [18].

Agar well diffusion assay

Manuka honey was purchased from ManukaPharm®, Leicestershire, UK with +20 Unique Manuka Factor (+20UMF) which contained approximately 800mg/kg MGO [19]. MGO is an active component of the Manuka honey which is formed by nectar derived dihydroxyacetone during ripening. Antibacterial activity of undiluted Manuka honey (+20UMF) was performed by agar well diffusion assay against NDM-1 positive *K. pneumoniae*, adopted from punch

plate assay. In short, 0.5McFarland bacterial suspension was inoculated on MHA plate and sterile 6mm cork borer used to make wells on each plate. Subsequently, 120μL undiluted Manuka honey was poured in each well and plates were incubated at 37°C overnight. Zone of inhibition (mm) was measured by Vernier caliber. The assay was performed in triplicate [20].

Minimum inhibitory concentration (MIC) of Manuka honey

Microbroth dilution assay was used to determine the MIC (%v/v) and minimum bactericidal concentration (MBC %v/v) of the Manuka honey [21]. Two to three isolated colonies were mixed in 20mL of double strength Lysogeny broth (LB) medium in 50mL of falcon tube and incubated at 37°C overnight. Bacterial suspension was diluted to achieve 0.5Mcfarland at OD of 0.07 at 600nm spectrophotometrically. Bacterial suspension was further diluted 1:100 with double strength LB broth to achieve a final concentration of 1x 10^5 CFU/mL. Briefly, serial dilutions of Manuka honey (5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% and 50%) were prepared in sterile distilled deionized water and 100μL of each honey dilution was added in 96-wells, flat bottom micro titer plates (Thermo Fisher Scientific, UK). Subsequently, 100μL of bacterial suspension was added into each well. Negative control wells contained 100μL of LB broth and positive control wells contained LB medium inoculated with bacteria suspension. Microtiter plate was incubated at 37°C overnight at shaking incubator (Max^{QTM} Mini 4450) at 3g. MIC was calculated by comparing each well with negative and positive control wells. All the procedure was performed in triplicate.

Minimum bactericidal concentration (MBC) determination

MBC is defined as first dilution with no growth on agar plate. A 10μL sample was taken from the no visible growth wells of microtiter plate and was inoculated on the nutrient agar plates (Oxoid, UK) and aerobically incubated at 37°C for 24 hours. Plates were examined for cell viability. Any colonies that developed were scored as bacterial growth and no bacterial growth. All the procedures repeated in triplicate.

Animals and experimental groups:

Before staring the clinical experiment, permission was taken by the Ethical Review Committee for Medical and Biomedical Research, University of Health Sciences, Lahore, Pakistan.

BALB/c mice between the ages of 12-14 weeks, weighing 30-35 grams were used from the Animal Facility, University of Health Sciences, Lahore, Pakistan. All the mice kept in cages containing sawdust and wood shavings as litter material. Animal room temperature was maintained at 25±2°C and provided with 12 hours light and dark cycles. They were given 14 days adaptation period and during this interval they were screened for any infections or abnormalities. They were kept on standard balance diet for rodents and fresh potable water *ad libitum* [22].

Experimental work for *in vivo* activity of Manuka honey

Mice were divided into five different experimental groups; A (negative control, non-infected mice), B (positive control, infected mice), C (infected mice treated with 20% Manuka honey), D (infected mice treated with 30% Manuka honey) and E (infected mice treated with tigecycline) and each group comprised of eight mice. Mice of Groups B, C, D and E were challenged with 100μL of 1×10^5 CFU of NDM-1 *K. pneumoniae* ST11 strain through lateral tail vein to produce systemic infection [23] and observed for any clinical signs and symptoms. However, Group B mice were not treated with any therapeutic agent and blood culture was performed to establish the bacteremia in BACTEC 9120 (Becton Dickinson Diagnostic Instrument System, Spark, Md) instrument. After 3 hours post infection, mice of Group C and D were treated with 200μL of 20% and 30% (v/v) Manuka honey through lateral tail vein respectively with an interval of 12 hours for next two weeks [11]. Blood culture was performed on 7th and 15th day post treatment for bacterial count (CFU). However, mice of Group E administered 5mg/kg intravenous injection of tigecycline (Pfizer, Laboratories, USA) twice a day after 3 hours post infection and treated for seven days [24] and blood culture was performed on seven days for CFU.

Blood culture and bacterial count

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Blood (0.1mL) was drawn from the lateral tail vein of each mice using aseptic technique. Blood was immediately inoculated into blood culture bottle (BD Peds plus/F) and processed in BACTEC 9120 (Becton Dickinson Diagnostic Instrument System, Spark, Md). Positive specimens were sub-cultured on blood agar and incubated at 37°C overnight. *K. pneumoniae* was re-identified using VITEK 2 system (BioMerieux, France). Bacteria (CFU) were calculated by Miles and Misra method.

Histological analysis

All the experimental mice (Group C and D) and control mice (Group A) were anesthetized and sacrificed according to standard operating procedures. Mice kidneys, liver and spleen were surgically removed and fixed in 10% formaldehyde, embedded in paraffin wax and stained with hematoxylin and eosin (H/E). Microscopic analysis was performed by the qualified histopathologist and reports were interpreted by scoring as mild, moderate and sever.

Statistical Analysis

All statistical analysis was done using statistical package for social sciences (SPSS) software (version 22). Numerical data were determined using mean ± SD. One-way ANOVA was applied to determine the mean differences (bacterial count) among the groups. Tukey test and Chi-square/ Fishers test were used to compare the categorical data among groups. *P* value ≤ 0.05 will be considered as significant.

Results

Molecular characterization and identification:

NDM-1 producing *Klebsiella pneumoniae spp pneumoniae* which belongs to the ST11 was confirmed after multi sequence alignment (Accession no. KY446367) and MLST analysis respectively. Moreover, plasmid characterization revealed that isolate contained five different sizes of plasmids (~80, 100, 150, 200 and 270 kb) and bla_{NDM} was located on 150kb and 270kb of plasmids. Furthermore, *K. pneumoniae* was also capable to produce carbapenamase and MBLs (Figure 1 and 2).

Antibiogram profiling:

MIC (μg/mL) of the *K. pneumoniae* displayed 100% resistance against β-lactam antibiotics and β-lactam inhibitors however, minocycline (8μg/mL) and trimethoprim (8μg/mL) exhibited intermediate resistance, while most effective drugs were colistin and tigecycline (≤0.5μg/mL) (Table 1).

***In vitro* efficacy of Manuka honey:**

Concentrated Manuka honey displayed limited antibacterial activity with an average zone of inhibition of 9mm± 0.0 SD by agar well diffusion assay (Figure 3). However, *K. pneumoniae* was inhibited and killed at 30% v/v of Manuka honey by microbroth dilution assay.

***In vivo* activity of Manuka honey:**

In general, mice of Group A (negative control) remained clinically healthy during the whole span of trial. However, after the bacterial inoculation, all the mice of Group B, C, D and E developed severity of clinical signs and symptoms of infection including lack of alertness, lack of movement, piloerection, conjunctival injection/mucky eyes and hunched backs. Following the treatment with antibiotic, all the mice of Group E were fully recovered after one week with no apparent clinical symptoms while substantial clinical improvement was observed in the mice of Group C and D post-treatment with 20% and 30% Manuka honey respectively. Furthermore, no mice mortality occurred during the clinical trial.

Mean log value for Group B (positive control) animals was 2.744 after one week of infection that was increased to 3.238 during the second week. However, mean log value for Group C (treated with 20% Manuka honey) mice was 1.829 after 7 days that was reduced to 1.035 at the end of second week. Similar trends were observed in Group D (treated with 30% Manuka honey) mice in which mean log value was substantially decreased from 1.624 to 0.610 in first and second week respectively (Figure 4). One-way ANOVA indicated statistically significant differences among treatment groups (F=74.326, *P*- <0.0001) and effects of interaction between treatment and mean duration period (F=8.614, *P*- <0.0001) which suggested that continuous treatment for extended period had negative effect on mean CFU count. However,

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no significant effect was observed on treatment period alone. Tukey (HSD) test showed significant differences among different mice groups. They were statistically highly significant (P- <0.0001) when compared to positive control (Group B). Similarly, CFU count from mice Group E (treated with antibiotic) was also statistically highly significant (P- <0.0001) when compared with Group B, C and D. However, no statistical difference was observed between mice Group C and D (P- 0.384).

Histopathological analysis of Manuka honey treated groups:

Liver of Group C and D mice

The liver cells of the Group A (negative control) animals had normal histological morphology however, hepatocytes of the Group C and D mice harboring lobules and trabeculae having round centrally placed nuclei and moderate amount of eosinophilic granular cytoplasm. While, moderate number of inflammatory cells infiltrated lobules especially around central vein which are predominantly composed of lymphocytes and few neutrophils. Moreover, few sinusoids were dilated along with lymphoid aggregates and focal areas of spotty necrosis were also evident. However, no portal tracts were seen (Figure 5). Overall, no distinctive cytological changes in liver cells of Group C and D mice were observed.

Kidney of Group C and D mice:

Renal cells of the control mice exhibited normal histology profile however, H/E section of the Group C and D mice revealed that interstitium showed mild infiltration of inflammatory cell including lymphocytes and few neutrophils. Furthermore, normal renal parenchyma composed of glomeruli and tubules while few glomeruli were sclerosed. Surrounding tubules were lined by cuboidal epithelial cells having regular nuclei and granular eosinophilic cytoplasm. In general, both the mice Groups displayed benign renal tissue with non-specific inflammation (Figure 6).

Spleen of Group C and D mice

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The spleen cells of the control group exhibited normal cellular morphology. Although, spleen of the Group C and D mice presented numerous scattered multinucleated giant cells, bizarre cells and increased mitotic activity. Areas of hemorrhage and sheets of lymphocytes were also noticed (Figure 7).

Discussion

Rapid dissemination of NDM producing pathogens is becoming a big challenge worldwide. Although Indian subcontinent is the main reservoir for NDM-1 producing *Enterobacteriaceae* but several cases also reported from the Balkan State. NDM gene is mainly located on plasmids and easily transferable among bacterial population. In present study, we characterized NDM-1 producing *K. pneumoniae* which belongs to the ST11. Similar findings have been documented from different regions including India, UK and Sweden [25, 26], USA [27] and Poland [28]. However, as per our knowledge we are first time reporting NDM-1 positive *K. pneumoniae* ST11 in Pakistan. Spread of this clinical pathogen in our clinical setting is mainly due to the poor health care management, unhygienic practices, overcrowded wards and hospitals, cross contamination due to sharing beds and use of contaminated intravenous catheter [29]. Genetic characterization of the isolate revealed presence of *bla*$_{NDM}$ on ~150kb and ~270kb plasmids. Previous studies from Bangladesh, India and UK documented the presence of *bla*$_{NDM}$ on the transmissible plasmids ranging from 80kb to 500kb in *Enterobacteriaceae* family [3, 30]. MIC of the isolate depicted high resistance not only against β-lactam drugs but also other classes of antibiotics and most effective drugs were colistin and tigecycline. These findings are in accordance with our previous study on the spread of NDM-1 in pediatric patients in Pakistan [4]. Furthermore, studies from Pakistan, India and UK also reported almost similar findings and colistin was the drug of choice [3, 5, 31]. This resistance is directly linked to the irrational and broad spectrum use of antibiotics in our clinical settings, other important factors includes self-medication and free access to antibiotics at pharmacy corner [29].

According to previous studies, Manuka honey exhibited bacteriogenic activity against different MDR pathogens including ESBL producing *K. pneumoniae* (30%-40%v/v) [32], *P. aeruginosa* (12%v/v) [33] and *A. baumannii* (12.5%v/v) [34]. In current study, 30%v/v Manuka honey is sufficient to inhibit and kill NDM-1 producing *K. pneumoniae* ST11. In our knowledge, there is no data available on the antibacterial activity of honey against the NDM-

1 positive *K. pneumoniae* ST11 so far. In this study, we have analyzed the *in vivo* activity of Manuka honey against the NDM-1 positive *K. pneumoniae* strain (Figure 4). As per our knowledge, there is no data available on the intravenous use of Manuka honey in mouse model infected with NDM positive *K. pneumoniae* strain so far. However, one of our previous study documented the intravenous administration of Manuka honey completely recovered the MRSA infected mice with no mouse mortality [11]. Moreover, Al-Waili et al reported the intravenous inoculation of honey in sheep which presented a significant improvement in lipid profile, renal profile and other proteins [35]. Intravenous injection of Manuka honey lead to substantial reduction of bacterial load in a time dependent manner in present study. Overall, 20%v/v honey treatment resulted in ≥1 log reduction of the bacterial CFU, while 30%v/v caused 1-2 log bacteria reduction. In comparison to study conducted on MRSA infected mice [11], Manuka honey treatment was unable to fully recover bacteremia induced by *K. pneumoniae* may be attributed to its stringent nature. Surprisingly, histopathological studies revealed mild to moderated morphological changes in liver, kidneys and spleen after honey treatment. In contrary, a study from Pakistan depicted that high oral dose of Manuka honey to treat MRSA leads to severe histological changes of the kidney cells including focal tubular epithelial cell degeneration and coagulative necrosis [36]. As per our knowledge there is no data available on the histological effect of Manuka honey against NDM producing *K. pneumoniae* ST11 so far.

Conclusion

This study conclude that Manuka honey has a potential effect on the NDM-1 producing *K. pneumoniae* both *in vitro* and *in vivo*. Therefore, Manuka honey can be used as a substitute or in combination with antibiotics to treat the infections caused by such pathogens after several clinical trials.

Acknowledgement

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Conflict of interest

There is no conflict of interest.

Table 1: MIC (μg/mL) of NDM-1 producing *K. pneumoniae* ST11

Isolate	SAM	PIP	CXM	CXA	CFM	CRO	FEP	ATM	MEM	LEV	MXF	MNO	TE	TMP	C	TGC	CS
	≤2 - ≥32	≤4 - ≥128	≤1 – ≥64	≤1 – ≥64	≤0.25 - ≥4	≤1 – ≥64	≤2 – ≥64	≤1 - ≥64	≤0.5 - ≥16	≤0.12- ≥8	≤0.25 - ≥8	≤1 - ≥16	≤1 - ≥16	≤0.5 - ≥16	≤2 - ≥64	≤0.5 - ≥8	≤0.5 - ≥16
NDM-1 producing *K. pneumoniae* ST11	≥32	≥128	≥64	≥64	≥4	≥64	≥64	≥64	≥16	≥8	≥8	8	≥16	8	4	≤0.5	≤0.5

SAM: Ampicillin/Sulbactam, PIP: Piperacillin, CXM: Cefuroxime, CXA: Cefuroxime Axetil, CFM: Cefixime, CRO: Ceftriaxone, FEP: Cefepime, ATM: Aztreonam, MEM: Meropenem, LEV: Levofloxacin, MXF: Moxifloxacin, MNO: Minocycline, TE: Tetracycline, TMP: Trimethoprim, C: Chloramphenicol, TGC: Tigecycline, CS: Colistin,
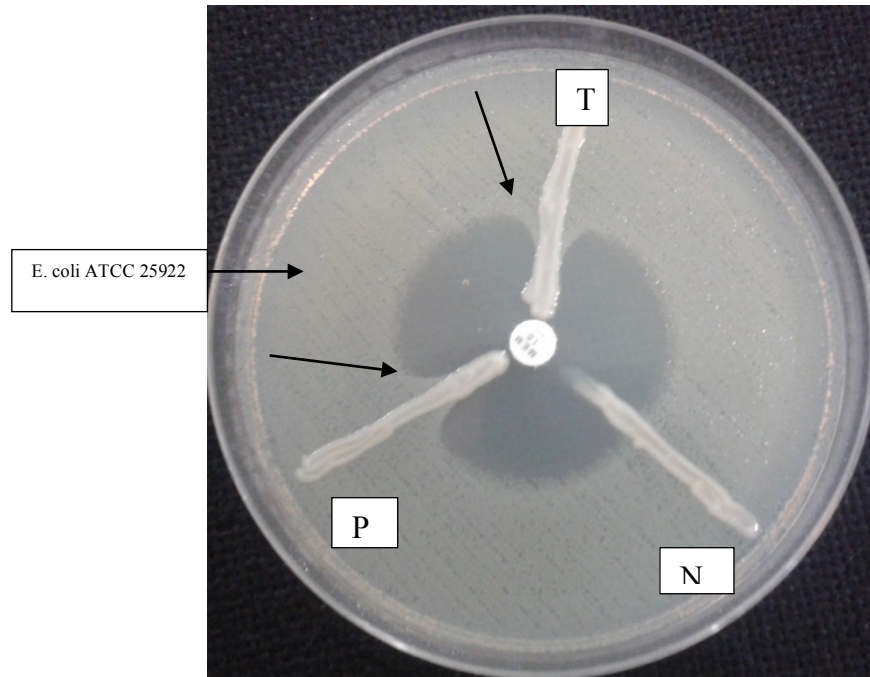
Figure 1: Modified Hodge's test for carbapenamase detection. Black arrows shows the cloverleaf like indetaion. T: test organism; P: positive control (known carbapenmase producing *K. pneumoniae*); N: negative control (*E. coli* ATCC 25922).
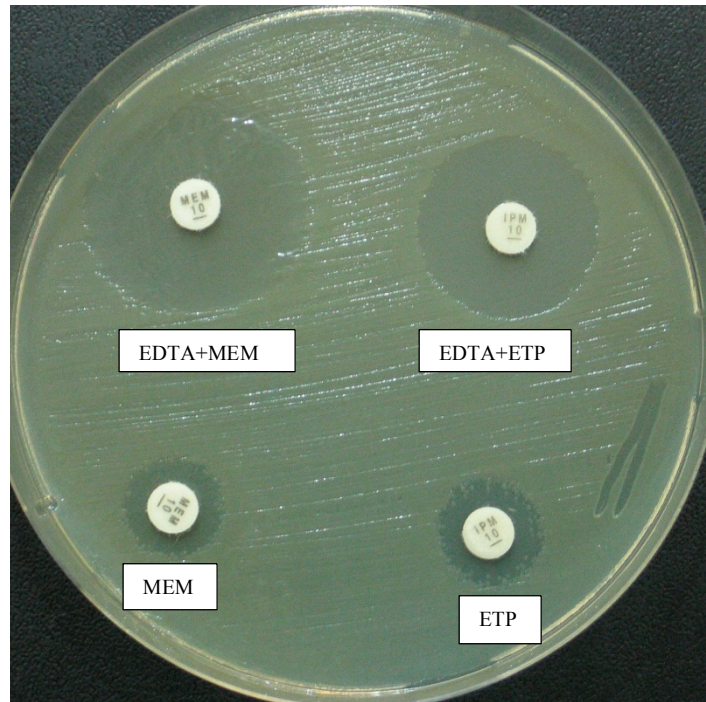
Figure 2: Double disc synergy method for MBLs detection. EDTA containing antibiotic discs shows >5mm zone of inhibition as compare to the non EDTA discs.
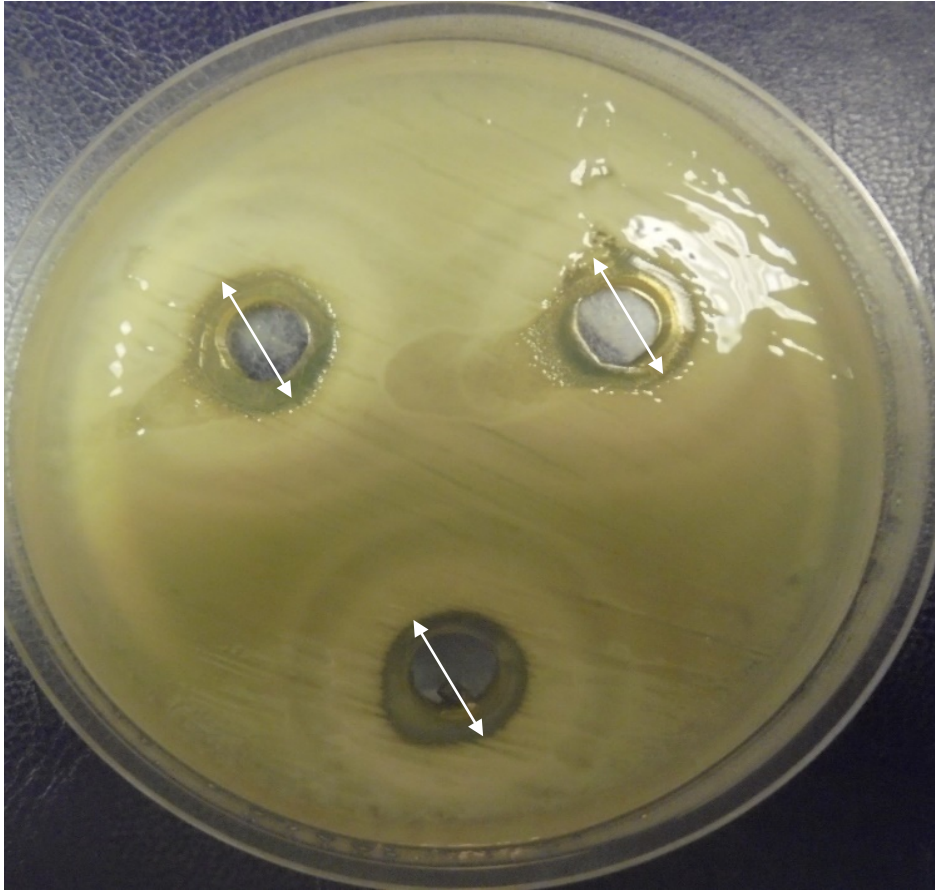
Figure 3: Agar well diffusion assay. White arrows indicate the zone of inhibition (mm) of Manuka honey against *K. pneumoniae* strain in triplicate.
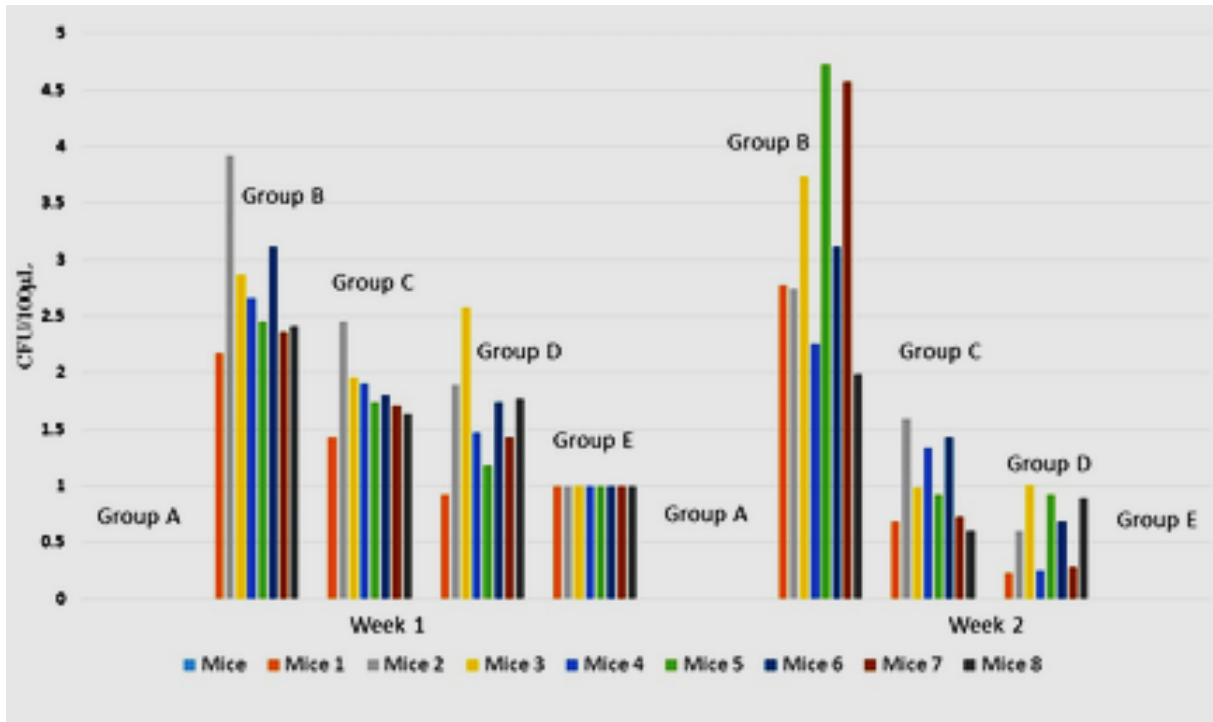
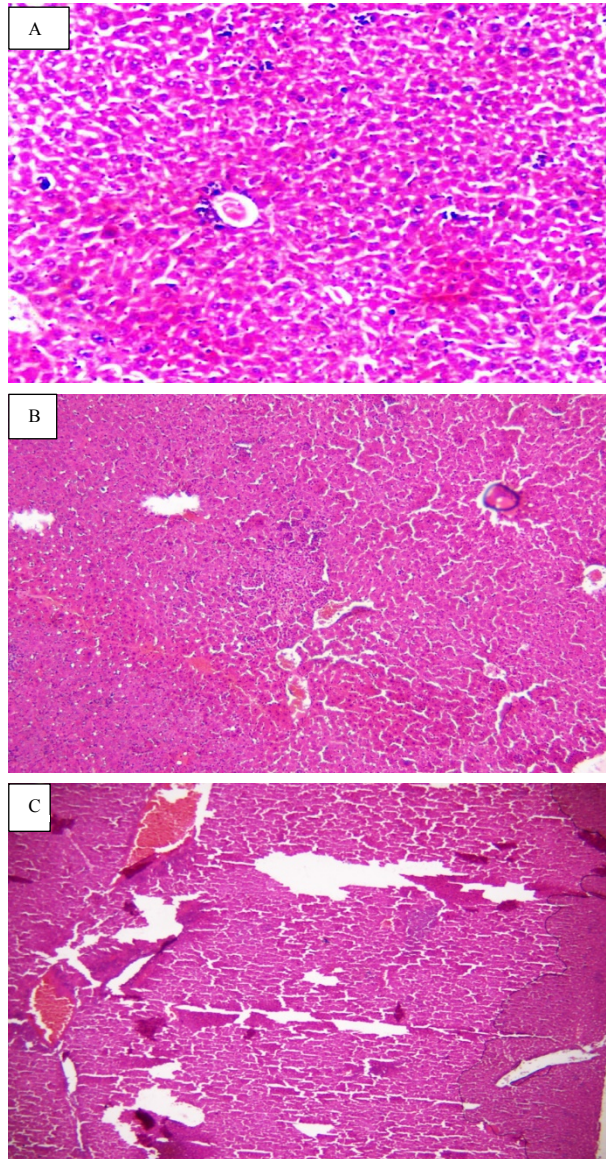**Figure 4** shows the first and second week of intravenous treatment of mice against different mice groups.

Figure 5: Histological analysis of mice hepatocytes; A: normal control, B: 20% Manuka honey; C: 30% Manuka honey (HPFx40).
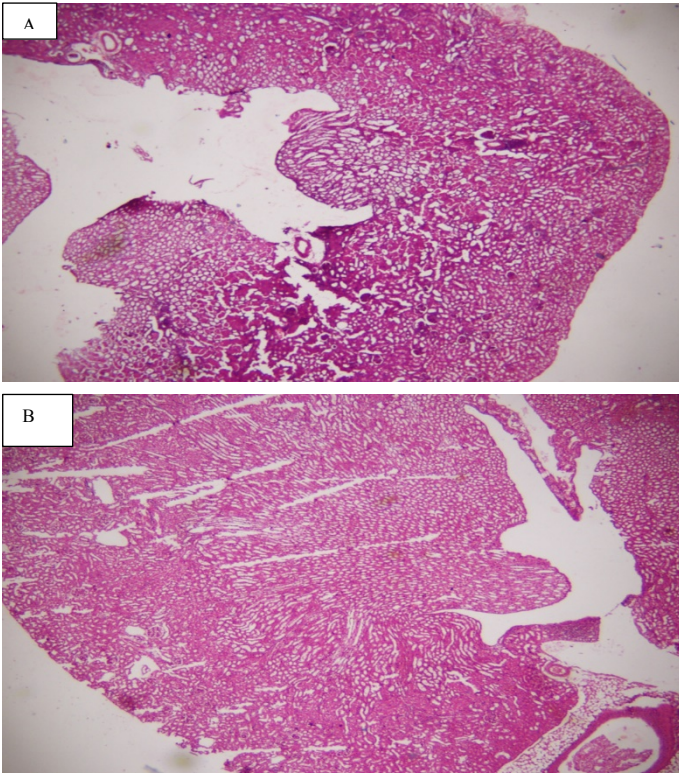
Figure 6: Histological examination of mice kidneys cells. A: 20% Manuka honey; B: 30% Manuka honey (HPFx40).
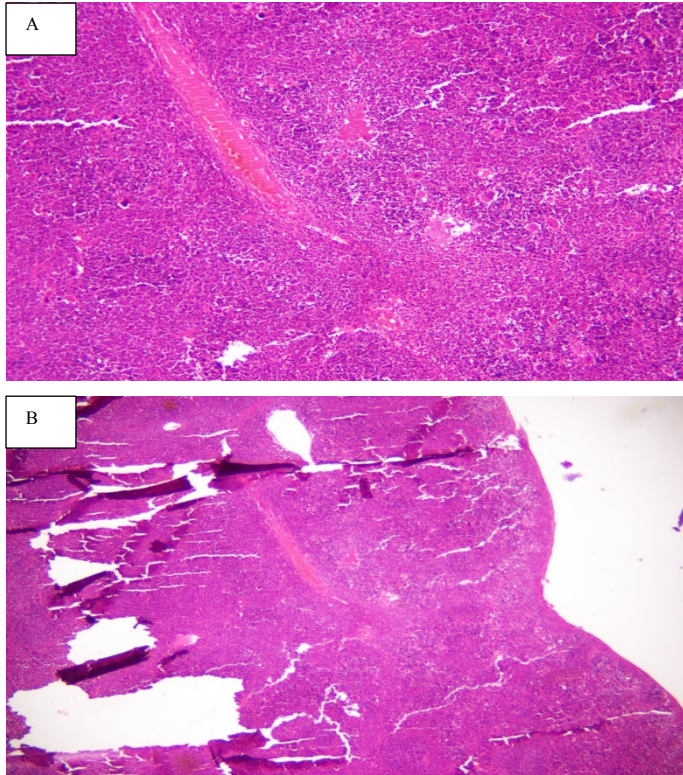
Figure 7: Histological results of mice spleen cells. A: 20% Manuka honey; B: 30% Manuka honey (HPFx40).

Summary points

- In developing countries, New Delhi metallo-β-lactamase producing *Klebsiella penumoniae* is a serious threat in public health sectors.

- These bacteria are difficult to treat due to the acquisition of bla_{NDM-1} which showed resistance against several antibiotics including carbapenems.

- Manuka honey is a medicinal honey widely used to treat MDR pathogens, hence, we first time used Manuka honey to treat such bugs *in vitro* as well *in vivo*.

Results:

- NDM-1 producing *K. pneumoniae* ST11 was confirmed and bla_{NDM-1} was located on 150kb and 270kb of plasmids.

- MIC (µg/mL) of the *K. pneumoniae* displayed 100% resistance to wide range of antibiotics including carbapenems and most effective drugs were tigecycline and colistin.

- *In vitro*, Manuka honey exhibited 9mm ±SD zone of inhibition while MIC (%v/v) and MBC (%v/v) was 30% against NDM-1 producing bug.

- *In vivo*, there was a substantial reduction of bacterial mean log (>1 log) following the two weeks of Manuka honey treatment while mild to moderate inflammation was observed in liver and spleen of the mice.

Conclusion:

The study determined that Manuka honey could be used as a therapeutic agent to treat NDM producing *K. pneumoniae* ST11 after several clinical trials *in vitro* and *in vivo*.

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Papers of special notes have been highlighted as: *of interest; ** of considerable interest

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