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Research Article
Prevalence, Characterization and Inhibition by Probiotics of Multidrug Resistant Bacteria Isolated from Renal Failure Patients Undergoing Hemodialysis

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Abstract

Background and Objectives: Characterization and inhibition by probiotics of multidrug resistant (MDR) bacteria causing renal failure patients and receiving hemodialysis were the target of this study. The prime objective of this study was to study the prevalence of MDR within Egyptian renal failure patients and to inhibit them by probiotics. **Materials and Methods:** The pathogenic bacteria were isolated from clinical samples and were then characterized by biochemical and molecular methods. Inhibition of MDR bacteria by cell free supernatants (CFS) from the probiotic *Enterococcus bacium* NM_2 (*E. faecium* NM_2) was studied *in vitro*. **Results:** One hundred bacterial isolates were isolated and into 76% Gram negative bacilli and 24% Gram positive cocci. Based on characterization of such isolates, 7 groups were found and could be arranged in the following descending order according to number of strains identified: *Escherichia coli (E. coli*, 35%) > *Klebsiella pneumoniae (K. pneumoniae*, 18%) > *Staphylococcus aureus* (*S. aureus*, 17%) > *Pseudomonas aeruginosa (P. aeruginosa*, 16%) > *Proteus vulgaris (Prot. vulgaris*, 8%) > *Staph. Saprophyticus* (4%) > *Streptococcus pyogenes (S. pyogenes*, 2%). Susceptibility of such bacteria to antibiotics was studied and the more resistant strains (4 strains) were characterized by 16S rRNA cataloging analysis. CFS obtained from the probiotic bacterium *E. faecium* NM_2 inhibited distinctively the growth of 4 MDR bacterial strains (RF22, RF27, RF51, RF55). **Conclusion:** One hundred bacterial isolates obtained from hemodialysis patients were isolated and identified herein. About 20% of such isolates were MDR. CFS from *E. faecium* NM2 inhibited the more MDR bacteria.

Key words: Probiotics, multidrug resistant (MDR) bacteria, hemodialysis, renal failure

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Due to the prevalence of MDR bacteria in hemodialysis patients suffering from disease complications, it is necessary to characterize such MDR bacteria and their inhibition is a great challenge. In this regard, this study was an endeavour to use probiotics as alternative inhibitory agents of MDR bacteria

Hemodialysis, commonly called kidney dialysis is a processes of purifying the blood of a person whose kidneys are not working normally; this is to remove waste products such as creatinine and urea[1]. Infections have been a major complications in hemodialysis patients due to their immune compromised systems and due to catheters changing continuously[2]

The UTIs infections are normally resulted in development of cystitis, pyelonephritis, urethritis, endometritis and other undiagnosed UTIs. Therefore, such infection complications are the second leading cause of death in the first year of hemodialysis patients[3].

It was found also that bacteremia/septicemia in hemodialysis patients is very high compared with its incidence in general population hemodialysis catheter uses were at higher risk of bacteremia[1,4]. This clearly shows that there is a need to continue research to characterise pathogenic bacteria obtained from hemodialysis patients, in general and that suffering from infection complications, in particular.

Antibiotic resistance in uropathogens is increasing worldwide; it varies according to geographic area and is due to many reasons such as misuse of antibiotics, microbial conjugation and gene(s) transfer among natural microflora of human body. The thickening of cell wall, production of enzymes by pathogenic bacteria and modifications of specific site(S) receptors necessary for antibiotic action[5]. The term MDR is used to describe bacteria that are able to resist the action of $\geq$3 antibiotics[6]; the prevalence of MDR in hemodialysis patients and with infection complication is dangerous problem and a high challenge to be controlled. This clearly showed that there is a mandatory need to search for natural agents to be mixed or combined with antibiotics to act in synergism and to inhibit the MDR bacteria[7-10].

It was found previously that there is an inversely proportion between probiotics and pathogenic bacteria in urine, other study showed that healthy men with healthy urogenital tract are rich with probiotics in their urine[11,12]. Hence probiotics isolated from urine showed promising use in bio-controlling UTIs[13]. The present work was undertaken to characterize and determine the prevalence of infections bacteria especially MDR bacteria in hemodialysis patients and to start work about their bio-control by probiotics.

MATERIALS AND METHODS

Collection of clinical samples: The subject population of this study was 100 patients from all age ranges; all of them were suffering from renal failure and other disease complications and are receiving long term hemodialysis at Hemodialysis Unit, Nephrology Department, Zagazig University Hospitals, Egypt. Microbiological cultures were orders by physicians 5 times at 3 days intervals from certain clinical samples including urine, urinary catheters, urinary dialysates and blood in the period from January 5 till December 20, 2014. Samples were taken and analyzed in Microbiology Lab., Zagazig University Hospitals, Egypt.

Isolation and purification of bacteria: The clinical specimens were streaked by sterile needle loops on petri dishes containing nutrient agar, blood agar and MacConkey agar (Oxoid) (3corner plates, Gomhoria Co., Egypt). After incubation at 37°C for 48 h, pure homogenous growth(s) were purified on the same media and after incubation for 48 h, single colonies were picked up by sterile needles and streaked onto slope cultures of the same media that were stored in refrigerator throughout the study period[14].

Antibiotic susceptibility test: One hundred pure bacterial isolates were obtained. They were analysed for their antibiotic susceptibility using 14 antibiotics was used the Kirby Bauer disc diffusion assay onto Muller Hintorn agar (Oxoid)[15,16].

Characterization and identification of the 100 bacterial isolates: The 100 bacterial isolates were characterized regarding Gram staining, cell morphology, catalase and oxidase reactions[16-19].

The identification was completed by API-kits (Biomerieux, France) according to the manufacturer's instructions. The identification of the more antibiotic resistant strains (MDR) (RF22, RF27, RF51, RF55) were confirmed using 16S rRNA fingerprinting. Total DNA(S) were extracted. The l6S rRNA gene(S) were amplified using PCR technique with specific primer 5^{-}-AGAGTTTGATCCTGGCTCAG-3^{-} as the foreward one and 3^{-}-TTCAGCATTGTTCCATTGGC-5^{-} as the reverse primer. The gene(s) amplifications were carried out as described previously[19,20].

The PCR products were cleaned up using Gene JET™ PCR purification kit (Fermentas) and were then sequenced at GATC Biotech AG (Konstanz, Germany) using ABI 3730X1 DNA sequencer. The sequences were submitted to Gene Bank under accession numbers MH 762086, MH 762087,

MH762088, MH762098 for bacterial isolates RF22, RF27, RF51, RF55 respectively, comparised to deposited data using Basic local Alignment Search Tool Programme at http://ncbi.nlm.nih.gov/blast[21]. Phylogenetic trees were constructed by Clusta 1X programme that indicated the similarities of the present experimental 16S rRNA fingerprints with that stored in Gene Bank.

Inhibition of MDR bacteria by CFS from *E. faecium* NM_2: *Enterococcus faecium* NM_2 was isolated from urine of healthy man (Enan *et al.*[13]) and inhibited bacterial pathogens. CFS was collected by centrifuging (10.000 rpm) cells of the NM_2 strain grown in MRS broth for 15 min. About 250 mL flasks, each containing 99 mL Brain Heart infusion broth (BHI broth, Oxoid) were treated with sterile CFS of the NM_2 strain, inoculated by the 4 MDR bacteria at 2×10^4 CFU mL^{-1} final concentration (RF22, RF27, RF51, RF55) and incubated at 37°C for 5 days[13,22]. At suitable time intervals, 1 mL aliquots were withdrawn aseptically, diluted and CFU mL^{-1} values were calculated[23,24].

Statistical analysis: All results were expressed by the mean of triplicates plus the standard error using ANOVA variance analysis throughout SAS software. The least significant differences were used at $p<0.05$.

RESULTS

Relation of ages and gender to the collected clinical samples: As given in Table 1, about 42 and 58% of them were males, females respectively. About 13, 39 and 48% of patients were, less than 40 years old, in the age range 40-60 years old, more than 60 years old respectively (Table 1).

Relation between sources of clinical samples and patient diagnosis: The preliminary diagnosis of disease complications from the whole 100 patients is given in Table 2. About 50, 20, 10 and 20 were specimens of urine, blood, urinary catheters, renal dialysate respectively (Table 2). In correlation between source of specimens and physician diagnosis, it was showed

Table 1: Clinical samples (%) collected from different ages and gender

	Male		Female		Total	
Age range	No.	%	No.	%	No.	%
<40 years	4	30.8	4	69.2	13	13
From 40-60 years	15	38.5	24	61.5	39	39
>60 years	23	47.9	25	52.1	48	48
Total	42		58		100	100

Table 2: Relation of the sources of clinical samples and patient diagnosis

Isolate code	Diagnosis	Source of clinical sample
RF1	UTIs	Urine
RF2	UTIs	Urine
RF3	Pyelonephritis	Urine
RF4	UTIs	Urine
RF5	UTIs	Urine
RF6	Pyelonephritis	Urine
RF7	Urethritis	Urine
RF8	UTIs	Urine
RF9	UTIs	Urine
RF10	UTIs	Urine
RF11	Pyelonephritis	Urine
RF12	Urethritis	Urine
RF13	Urethritis	Urine
RF14	Urethritis	Urine
RF15	Pyelonephritis	Urine
RF16	Cystitis	Urine
RF17	Cystitis	Urine
RF18	Pyelonephritis	Urine
RF19	Urethritis	Urine
RF20	UTIs	Urine
RF21	UTIs	Urine
RF22	UTIs	Urine
RF23	UTIs	Urine
RF24	UTIs	Urine
RF25	Urethritis	Urine
RF26	Urethritis	Urine
RF27	Cystitis	Urine
RF28	Cystitis	Urine
RF29	Cystitis	Urine
RF30	Diabetics	Urine
RF31	Pyelonephritis	Urine
RF32	Pyelonephritis	Urine
RF33	Cystitis	Urine
RF34	Diabetics	Urine
RF35	Diabetics	Urine
RF36	UTIs	Urine
RF37	UTIs	Urine
RF38	UTIs	Urine
RF39	Pyelonephritis	Urine
RF40	UTIs	Urine
RF41	Diabetics	Urine
RF42	Diabetics	Urine
RF43	Pyelonephritis	Urine
RF44	UTIs	Urine
RF45	UTIs	Urine
RF46	UTIs	Urine
RF47	Cystitis	Urine
RF48	UTIs	Urine
RF49	Urethritis	Urine
RF50	Pyelonephritis	Urine
RF51	Septicemia	Blood
RF52	Septicemia	Blood
RF53	Bacteremia	Blood
RF54	Septicemia	Blood
RF55	Fever	Blood
RF56	Fever	Blood
RF57	Septicemia	Blood
RF58	Fever	Blood
RF59	Chronic fever	Blood
RF60	Bacteremia	Blood

Table 2: Continued

Isolate code	Diagnosis	Source of clinical sample
RF61	Chronic fever	Blood
RF62	Chronic fever	Blood
RF63	Septicemia	Blood
RF64	Bacteremia	Blood
RF65	Fever	Blood
RF66	Fever	Blood
RF67	Chronic fever	Blood
RF68	Septicemia	Blood
RF69	Septicemia	Blood
RF70	Septicemia	Blood
RF71	Chronic retention	Catheters
RF72	UTIs	Catheters
RF73	Cystitis	Catheters
RF74	Chronic retention	Catheters
RF75	UTIs	Catheters
RF76	Pyelonephritis	Catheters
RF77	Chronic retention	Catheters
RF78	Urethritis	Catheters
RF79	UTIs	Catheters
RF80	Cystitis	Catheters
RF81	Diabetics	Used dialysate
RF82	Diabetics	Used dialysate
RF83	Chronic retention	Used dialysate
RF84	UTIs	Used dialysate
RF85	Urethritis	Used dialysate
RF86	Pyelonephritis	Used dialysate
RF87	Liver cirrhosis	Used dialysate
RF88	Chronic retention	Used dialysate
RF89	Cystitis	Used dialysate
RF90	Urethritis	Used dialysate
RF91	Cystitis	Used dialysate
RF92	Chronic retention	Used dialysate
RF93	Pyelonephritis	Used dialysate
RF94	UTIs	Used dialysate
RF95	Diabetics	Used dialysate
RF96	Liver cirrhosis	Used dialysate
RF97	Urethritis	Used dialysate
RF98	Cystitis	Used dialysate
RF99	Diabetics	Used dialysate
RF100	Liver cirrhosis	Used dialysate

that the 50 patients subjected to urine analysis (RF1-50) were categorized to 20, 10, 8, 7 and 5 general UTIs patients, pyelonephritis patients, urethritis patients, diabetics patients (Table 2). Blood cultures were ordered from 20 patients (RF51-70) also were suffering from bacteremia/septicemia (11 ones) and fever (9 patients). In addition, microbiological cultures were carried out from urinary catheters (patients from RF71- RF80) and urinary dialysates (patients from RF81-RF100); those patients were suffering from many complications such as undiagnosed UTIs, cystitis, chronic retention, urethritis and/or liver cirrhosis (Table 2).

Distribution of bacterial isolates according to their gram staining: Of the 100 bacterial isolates obtained, 76 and 24% were Gram negative, Gram positive isolates respectively

Table 3: Distribution of bacterial isolates according to their gram stain reaction and source of isolation

	Gram positive		Gram negative		Total	
Source of isolation	No.	%	No.	%	No.	%
Urine	6	12	44	88	50	50
Urinary catheters	3	30	7	70	10	10
Blood	10	50	10	50	20	20
Used dialysate	5	25	15	75	20	20
Total	24		76		100	100

Table 4: Susceptibility of bacterial isolates to different antibiotics

Antibiotics	Resistant (%)	Intermediate (%)	Susceptible (%)
Oxacillin	82	3	15
Cephalothin	76	11	13
Sulphamethoxazole/trimethoprim	73	9	18
Amoxicillin/clavulanic acid	68	16	16
Cefaclor	66	10	24
Azithromycin	58	10	32
Ampicillin/sulbactam	57	13	30
Vancomycin	53	10	37
Ceftriaxone	52	16	32
Ciprofloxacin	26	12	62
Nitrofurantoin	24	12	64
Ofloxacin	22	10	68
Amikacin	18	11	71
Imipenem	6	7	87

(Table 3). The 76 Gram negative isolates were isolated from urine (44), urinary used dialysate (15), blood (10), urinary catheters (7); however, the 24 Gram positive isolates were isolated from blood (10), urine (6), urinary used dialysate (5) and urinary catheters (3) (Table 3).

Susceptibility of bacterial isolates to antibiotics: Antibiotic susceptibility test was carried out for the 100 bacterial isolates obtained. Results were given in Table 4. The isolated bacterial pathogens were more susceptible to imipenem (87%), amikacin (71), ofloxacin (68%), nitrofurantoin (64%), ciprofloxacin (62%) and this is coupled with low resistance values of about 6, 18, 22, 24 and 26%, respectively and a rest values of about 7, 11, 10, 12 and 12% were intermediate respectively. The low values of susceptibility of organisms were detected with oxacillin (15%), cephalothin (13%), sulphamethoxazole/trimethoprim (18%), amoxicillin/ clavulanic acid (16%) which were correlated with higher resistance values of about 82, 76, 73 and 68%, respectively (Table 4). Other antibiotics were of moderate values regarding either susceptibility or resistance of pathogenic bacteria to them. The percentage of antibiotics resistance within the 100 bacterial isolates studied was of about 20% as 20 bacterial isolates were MDR bacteria (Table 5); they resisted the action of antibiotics used.

Table 5: Identified MDR bacteria, diagnosis of patient from which clinical samples were withdrawn and their antibiotic susceptibility profile

	Inhibition zone (mm)															
Codes	VA	CRO	AK	CEC	OFX	CL	OX	CIP	AMC	F	SXT	AZM	SAM	IPM	Identified bacteria	Diagnosis
RF5	R (9)	I (15)	R (12)	R (4)	S (27)	R (12)	R (9)	S (23)	I (15)	S (19)	R (8)	S (22)	R (9)	S (23)	*E. coli*	UTIs
RF19	R (8)	R (6)	S (19)	R (9)	I (16)	I (17)	R (4)	I (20)	R (7)	R (2)	S (18)	R (8)	S (22)	S (23)	*E. coli*	Urethritis
RF21	S (19)	I (17)	S (17)	R (9)	S (27)	R (7)	R (7)	S (22)	R (9)	R (9)	R (8)	R (11)	S (15)	S (23)	*P. aeruginosa*	UTIs
RF22	I (17)	S (21)	R (5)	I (17)	R (5)	R (12)	S (22)	R (7)	I (15)	R (3)	R (8)	S (23)	I (13)	R (10)	*P. aeruginosa*	UTIs
RF27	I (17)	R (12)	R (8)	R (9)	S (27)	S (25)	R (7)	R (7)	I (16)	I (16)	S (22)	R (11)	I (13)	R (11)	*E. coli*	Cystitis
RF33	R (5)	S (23)	R (6)	R (5)	S (27)	R (9)	R (7)	S (22)	R (9)	S (23)	S (23)	S (23)	R (11)	S (24)	*E. coli*	Cystitis
RF47	I (17)	R (10)	I (15)	I (16)	S (29)	R (6)	R (5)	R (2)	R (9)	R (9)	S (23)	S (24)	R (11)	S (24)	*K. pneumonia*	Cystitis
RF51	I (15)	R (12)	R (8)	R (9)	S (27)	S (25)	R (7)	R (7)	I (16)	I (16)	S (22)	R (11)	I (13)	R (11)	*K. pneumonia*	Septicemia
RF55	I (17)	S (21)	R (7)	I (16)	R (5)	R (10)	S (22)	R (7)	I (15)	R (3)	R (8)	S (23)	I (13)	R (6)	*S. aureus*	Fever
RF71	S (19)	R (12)	S (17)	R (9)	S (27)	R (8)	S (27)	S (27)	R (9)	S (23)	R (8)	I (17)	R (9)	I (15)	*P. aeruginosa*	Chronic retention
RF72	I (16)	R (12)	S (19)	S (19)	R (9)	R (12)	R (7)	R (2)	R (4)	S (21)	I (14)	R (9)	R (11)	S (23)	*P. aeruginosa*	UTIs
RF81	S (22)	S (24)	I (16)	R (9)	I (16)	R (2)	S (21)	S (27)	I (15)	S (23)	R (8)	I (17)	S (21)	S (23)	*S. aureus*	Diabetics
RF85	R (3)	I (16)	S (22)	R (6)	I (16)	I (16)	R (6)	S (22)	I (14)	R (13)	R (8)	I (17)	R (11)	S (23)	*E. coli*	Urethritis
RF88	R (3)	R (12)	I (16)	S (25)	S (29)	R (5)	R (7)	S (22)	R (9)	S (23)	I (14)	R (12)	R (8)	S (23)	*K. pneumonia*	Chronic retention
RF91	R (3)	S (21)	S (22)	I (15)	S (27)	R (7)	R (7)	I (19)	R (8)	R (9)	R (7)	I (17)	R (5)	S (25)	*E. coli*	Cystitis
RF93	R (6)	S (27)	I (16)	R (6)	S (27)	R (7)	R (7)	S (24)	I (15)	S (23)	R (9)	S (25)	R (11)	S (24)	*P. aeruginosa*	Pyelonephritis
RF97	R (3)	R (12)	S (22)	S (23)	S (29)	R (7)	R (8)	I (19)	R (9)	S (20)	R (9)	I (15)	S (20)	S (23)	*P. aeruginosa*	Urethritis
RF98	R (3)	S (21)	S (22)	I (15)	S (27)	S (23)	R (7)	R (8)	R (6)	I (16)	R (8)	S (23)	R (9)	I (15)	*S. aureus*	Cystitis
RF99	I (11)	S (22)	I (16)	R (9)	R (9)	R (7)	R (8)	R (4)	R (9)	S (23)	R (5)	S (25)	S (21)	S (23)	*P. aeruginosa*	Diabetics
RF100	S (19)	R (12)	S (21)	S (22)	S (27)	R (9)	S (27)	S (22)	R (9)	R (9)	S (18)	R (8)	I (13)	S (23)	*E. coli*	Liver cirrhosis

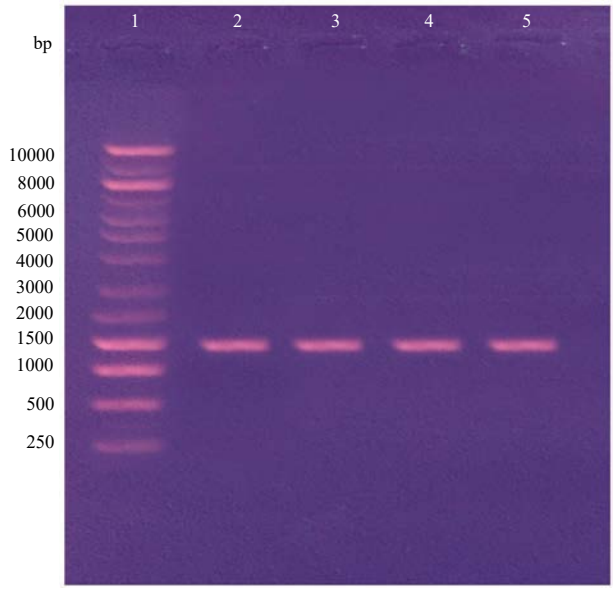
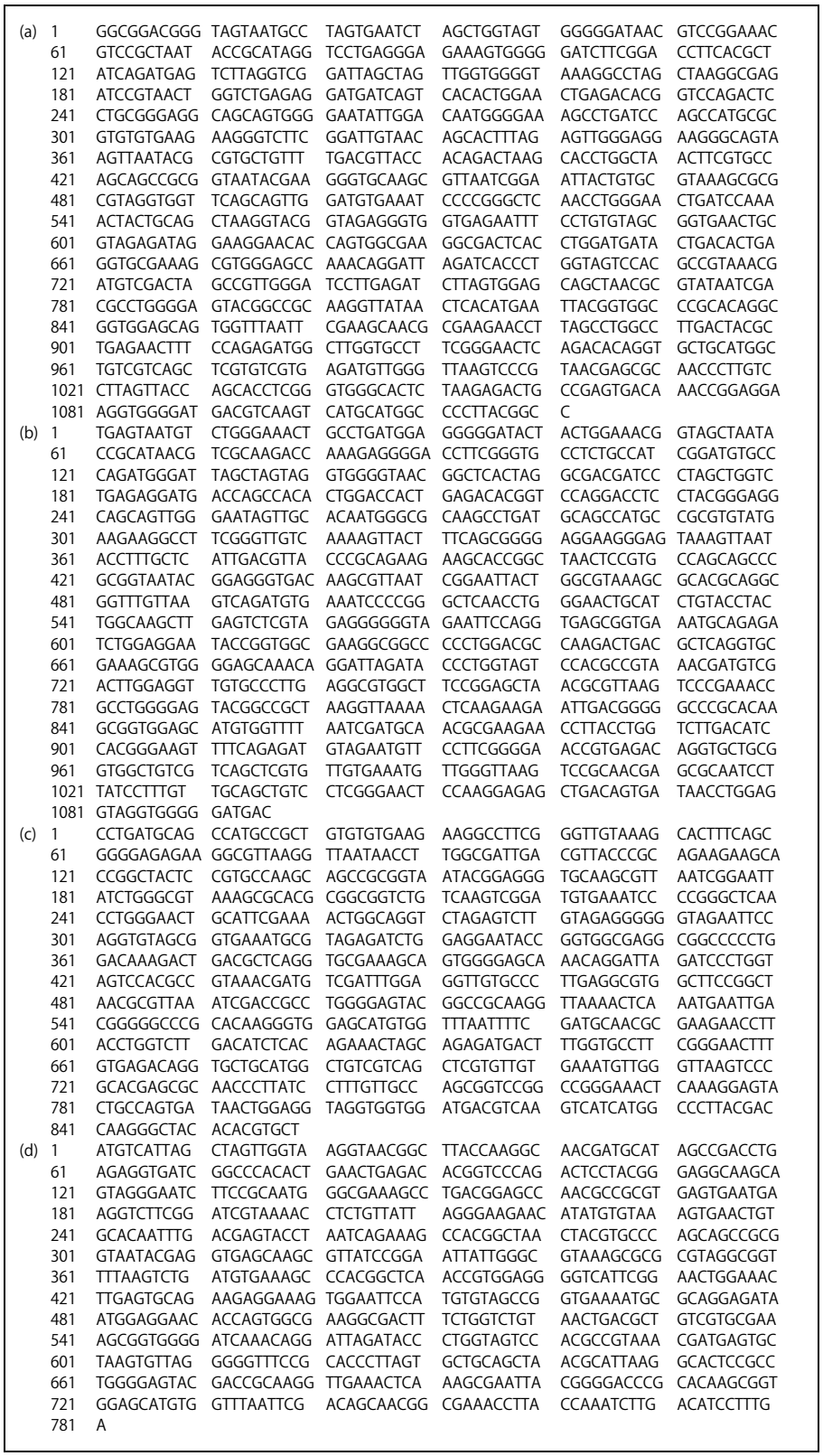


Fig. 1: Agarose gel electrophoresis of PCR products of 16S rRNA gene(s)
L: Lane, 1: DNA marker, 2: RF22 strain, 3: RF27, 4: RF51, 5: R55

Identification of bacterial isolates: The 100 bacterial pathogens were subjected to identification testes using API- kits (Biomerieux, France). According to the results obtained, the 100 bacterial isolates were classified into 7 groups which could be arranged in the following descending order according to the number of identified strains: *E. coli* (Group1, 35 strains)>*K. pneumoniae* (group 2, 18 strains)>*S. aureus* (group 3, 17 isolates)>*P. aeruginosa* (group 4, 16 isolates)>*Proteus vulgaris* (group 5, 8 isolates) >*S. saprophyticus* (group 6, 4 strains)>*S. pyogenes* (group 7, 2 strains). Out of the 100 bacterial isolates identified, 20 only were MDR. It is noted that the prevalence values of MDR bacteria identified were 7, 7, 3 and 3% for *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus* within the 100 identified bacterial strains.

Molecular identification of the more resistant bacteria to antibiotics: To confirm the biochemical identification that carried out by API-kits for the MDR bacteria, the more resistant strains were choosed from each 4 MDR bacterial groups viz-isolates RF22, RF27, RF51, RF55 and were subjected to molecular identification using 16S rRNA gene(S) fingerprints. DNA(s) were isolated from the four strains and 16S rRNA gene(S) was amplified using PCR technique. The PCR products were electrophoresed using agarose gel and indicated a successful amplification (1500 bp for each) (Fig. 1). DNA bands indicating 16S rRNA gene(S) were sequenced and the sequences (Fig. 2) were submitted to Gene Bank under accession numbers: MH762086, MH762087, MH762088 and MH762089 referring to isolates RF22, RF27, RF51, RF55 respectively. Using the Basic Local Alignment Search Programme (BLAST) phylogenetic trees and cluster analysis (Fig. 3a-d) were designed for each bacterial isolate and indicated that these isolates belonged *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *S. aureus* and designated *P. aeruginosa* RF22, *E. coli* RF27, *K. pneumonia* RF51 and *S. aureus* RF55, respectively.



(a)
1 GGCGGACGGG TAGTAATGCC TAGTGAATCT AGCTGGTAGT GGGGGATAAC GTCCGGAAAC
61 GTCCGCTAAT ACCGCATAGG TCCTGAGGGA GAAAGTGGGG GATCTTCGGA CCTTCACGCT
121 ATCAGATGAG TCTTAGGTCG GATTAGCTAG TTGGTGGGGT AAAGGCCTAG CTAAGGCGAG
181 ATCCGTAACT GGTCTGAGAG GATGATCAGT CACACTGGAA CTGAGACACG GTCCAGACTC
241 CTGCGGGAGG CAGCAGTGGG GAATATTGGA CAATGGGGAA AGCCTGATCC AGCCATGCGC
301 GTGTGTGAAG AAGGGTCTTC GGATTGTAAC AGCACTTTAG AGTTGGGAGG AAGGGCAGTA
361 AGTTAATACG CGTGCTGTTT TGACGTTACC ACAGACTAAG CACCTGGCTA ACTTCGTGCC
421 AGCAGCCGCG GTAATACGAA GGGTGCAAGC GTTAATCGGA ATTACTGTGC GTAAAGCGCG
481 CGTAGGTGGT TCAGCAGTTG GATGTGAAAT CCCCGGGCTC AACCTGGGAA CTGATCCAAA
541 ACTACTGCAG CTAAGGTACG GTAGAGGGTG GTGAGAATTT CCTGTGTAGC GGTGAACTGC
601 GTAGAGATAG GAAGGAACAC CAGTGGCGAA GGCGACTCAC CTGGATGATA CTGACACTGA
661 GGTGCGAAAG CGTGGGAGCC AAACAGGATT AGATCACCCT GGTAGTCCAC GCCGTAAACG
721 ATGTCGACTA GCCGTTGGGA TCCTTGAGAT CTTAGTGGAG CAGCTAACGC GTATAATCGA
781 CGCCTGGGGA GTACGGCCGC AAGGTTATAA CTCACATGAA TTACGGTGGC CCGCACAGGC
841 GGTGGAGCAG TGGTTTAATT CGAAGCAACG CGAAGAACCT TAGCCTGGCC TTGACTACGC
901 TGAGAACTTT CCAGAGATGG CTTGGTGCCT TCGGGAACTC AGACACAGGT GCTGCATGGC
961 TGTCGTCAGC TCGTGTCGTG AGATGTTGGG TTAAGTCCCG TAACGAGCGC AACCCTTGTC
1021 CTTAGTTACC AGCACCTCGG GTGGGCACTC TAAGAGACTG CCGAGTGACA AACCGGAGGA
1081 AGGTGGGGAT GACGTCAAGT CATGCATGGC CCCTTACGGC C

(b)
1 TGAGTAATGT CTGGGAAACT GCCTGATGGA GGGGGATACT ACTGGAAACG GTAGCTAATA
61 CCGCATAACG TCGCAAGACC AAAGAGGGGA CCTTCGGGTG CCTCTGCCAT CGGATGTGCC
121 CAGATGGGAT TAGCTAGTAG GTGGGGTAAC GGCTCACTAG GCGACGATCC CTAGCTGGTC
181 TGAGAGGATG ACCAGCCACA CTGGACCACT GAGACACGGT CCAGGACCTC CTACGGGAGG
241 CAGCAGTTGG GAATAGTTGC ACAATGGGCG CAAGCCTGAT GCAGCCATGC CGCGTGTATG
301 AAGAAGGCCT TCGGGTTGTC AAAAGTTACT TTCAGCGGGG AGGAAGGGAG TAAAGTTAAT
361 ACCTTTGCTC ATTGACGTTA CCCGCAGAAG AAGCACCGGC TAACTCCGTG CCAGCAGCCC
421 GCGGTAATAC GGAGGGTGAC AAGCGTTAAT CGGAATTACT GGCGTAAAGC GCACGCAGGC
481 GGTTTGTTAA GTCAGATGTG AAATCCCCGG GCTCAACCTG GGAACTGCAT CTGTCACTAC
541 TGGCAAGCTT GAGTCTCGTA GAGGGGGGTA GAATTCCAGG TGAGCGGTGA AATGCAGAGA
601 TCTGGAGGAA TACCGGTGGC GAAGGCGGCC CCCTGGACGC CAAGACTGAC GCTCAGGTGC
661 GAAAGCGTGG GGAGCAAACA GGATTAGATA CCCTGGTAGT CCACGCCGTA AACGATGTCG
721 ACTTGGAGGT TGTGCCCTTG AGGCGTGGCT TCCGGAGCTA ACGCGTTAAG TCCCGAAACC
781 GCCTGGGGAG TACGGCCGCT AAGGTTAAAA CTCAAGAAGA ATTGACGGGG GCCCGCACAA
841 GCGGTGGAGC ATGTGGTTTT AATCGATGCA ACGCGAAGAA CCTTACCTGG TCTTGACATC
901 CACGGGAAGT TTTCAGAGAT GTAGAATGTT CCTTCGGGGA ACCGTGAGAC AGGTGCTGCG
961 GTGGCTGTCG TCAGCTCGTG TTGTGAAATG TTGGGTTAAG TCCGCAACGA GCGCAATCCT
1021 TATCCTTTGT TGCAGCTGTC CTCGGGAACT CCAAGGAGAG CTGACAGTGA TAACCTGGAG
1081 GTAGGTGGGG GATGAC

(c)
1 CCTGATGCAG CCATGCCGCT GTGTGTGAAG AAGGCCTTCG GGTTGTAAAG CACTTTCAGC
61 GGGGAGAGAA GGCGTTAAGG TTAATAACCT TGGCGATTGA CGTTACCCGC AGAAGAAGCA
121 CCGGCTACTC CGTGCCAAGC AGCCGCGGTA ATACGGAGGG TGCAAGCGTT AATCGGAATT
181 ATCTGGGCGT AAAGCGCACG CGGCGGTCTG TCAAGTCGGA TGTGAAATCC CCGGGCTCAA
241 CCTGGGAACT GCATTCGAAA ACTGGCAGGT CTAGAGTCTT GTAGAGGGGG GTAGAATTCC
301 AGGTGTAGCG GTGAAATGCG TAGAGATCTG GAGGAATACC GGTGGCGAGG CGGCCCCCTG
361 GACAAAGACT GACGCTCAGG TGCGAAAGCA GTGGGGAGCA AACAGGATTA GATCCCTGGT
421 AGTCCACGCC GTAAACGATG TCGATTTGGA GGTTGTGCCC TTGAGGCGTG GCTTCCGGCT
481 AACGCGTTAA ATCGACCGCC TGGGGAGTAC GGCCGCAAGG TTAAAACTCA AATGAATTGA
541 CGGGGGCCCG CACAAGGGTG GAGCATGTGG TTTAATTTTC GATGCAACGC GAAGAACCTT
601 ACCTGGTCTT GACATCTCAC AGAAACTAGC AGAGATGACT TTGGTGCCTT CGGGAACTTT
661 GTGAGACAGG TGCTGCATGG CTGTCGTCAG CTCGTGTTGT GAAATGTTGG GTTAAGTCCC
721 GCACGAGCGC AACCCTTATC CTTTGTTGCC AGCGGTCCGG CCGGGAAACT CAAAGGAGTA
781 CTGCCAGTGA TAACTGGAGG TAGGTGGTGG ATGACGTCAA GTCATCATGG CCCTTACGAC
841 CAAGGGCTAC ACACGTGCT

(d)
1 ATGTCATTAG CTAGTTGGTA AGGTAACGGC TTACCAAGGC AACGATGCAT AGCCGACCTG
61 AGAGGTGATC GGCCCACACT GAACTGAGAC ACGGTCCCAG ACTCCTACGG GAGGCAAGCA
121 GTAGGGAATC TTCCGCAATG GGCGAAAGCC TGACGGAGCC AACGCCGCGT GAGTGAATGA
181 AGGTCTTCGG ATCGTAAAAC CTCTGTTATT AGGGAAGAAC ATATGTGTAA AGTGAACTGT
241 GCACAATTTG ACGAGTACCT AATCAGAAAG CCACGGCTAA CTACGTGCCC AGCAGCCGCG
301 GTAATACGAG GTGAGCAAGC GTTATCCGGA ATTATTGGGC GTAAAGCGCG CGTAGGCGGT
361 TTTAAGTCTG ATGTGAAAGC CCACGGCTCA ACCGTGGAGG GGTCATTCGG AACTGGAAAC
421 TTGAGTGCAG AAGAGGAAAG TGGAATTCCA TGTGTAGCCG GTGAAAATGC GCAGGAGATA
481 ATGGAGGAAC ACCAGTGGCG AAGGCGACTT TCTGGTCTGT AACTGACGCT GTCGTGCGAA
541 AGCGGTGGGG ATCAAACAGG ATTAGATACC CTGGTAGTCC ACGCCGTAAA CGATGAGTGC
601 TAAGTGTTAG GGGGTTTCCG CACCCTTAGT GCTGCAGCTA ACGCATTAAG GCACTCCGCC
661 TGGGGAGTAC GACCGCAAGG TTGAAACTCA AAGCGAATTA CGGGGACCCG CACAAGCGGT
721 GGAGCATGTG GTTTAATTCG ACAGCAACGG CGAAACCTTA CCAAATCTTG ACATCCTTTG
781 A

Fig. 2(a-d): Sequences of the 16S rRNA genes of (a) *P. aeruginosa* RF22, (b) *E. coli* RF27, (c) *K. pneumoniae* RF51 and (d) *S. aureus* RF55

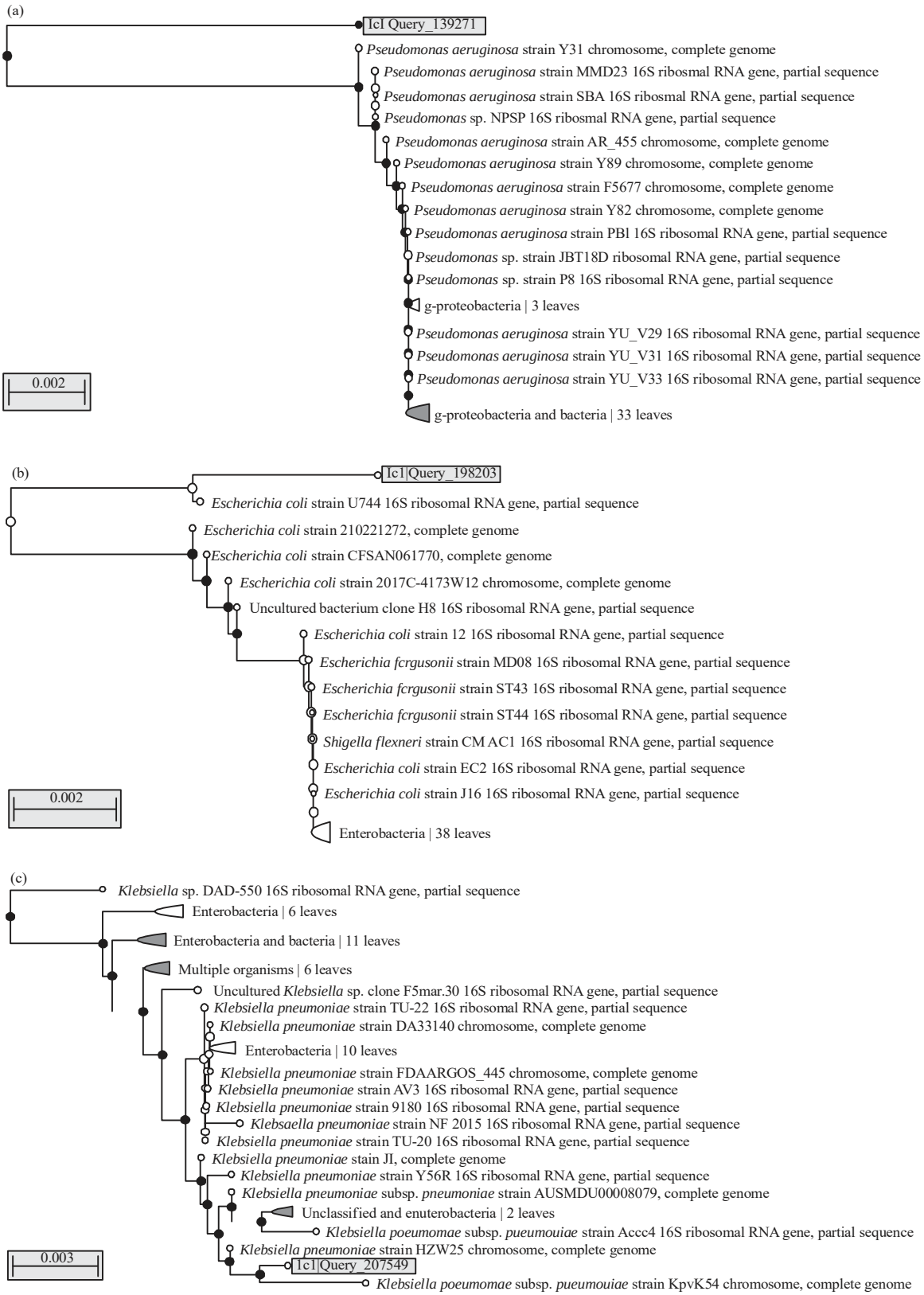


Fig. 3(a-d): Continued

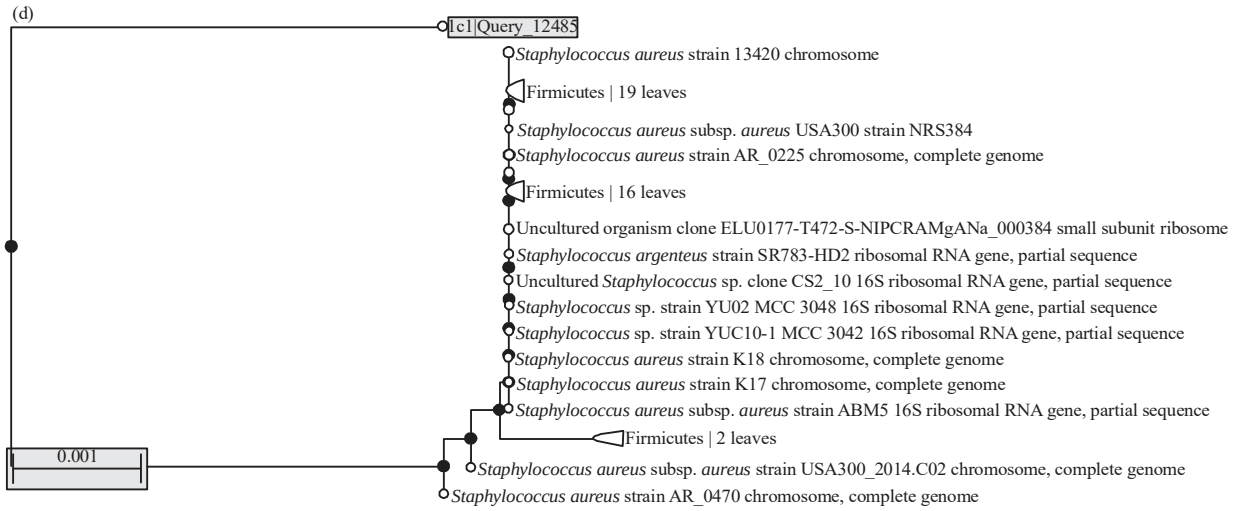


Fig. 3(a-d): Phylogenetic tree of (a) *P. aeruginosa* RF22, (b) *E. coli* RF27, (c) *K. pneumoniae* RF51 and (d) *S. aureus* RF55

Inhibition of MDR bacteria by CFS(s) obtained from *E. faecium* NM_2: CFS(s) were collected from *E. faecium* NM_2 and were added (1%) to cell suspensions of the MDR bacteria. Results are given in Fig. 4a-c and d). The control cells were increased step wisely, reaching either >6 log cycles in both the strains FR22 and RF51 or >8 log cycles in case of the strains RF27 or RF55. The treated MDR bacteria by CFS (s) of the probiotic bacterium *E. faecium* NM_2 decreased distinctively ($p<0.03$) and difference between control growth and growth of treated cells was almost 3 log cycles in case of *P. aeruginosa* RF22, *E. coli* RF27, *K. pnemoniae* RF5, no growth of *S. aureus* RF55 was found after 48 h of incubation.

DISCUSSION

The study population (100 patients) was chosen as they were renal failure patients and undergoing hemodialysis and suffering from some disease complications as diagnosed by physicians such as cystitis pyelonephritis, bacteremia urethritis and general UTIs and two persons were liver cirrhosis patients; hence 100% of cultures ordered by physician were positive. This showed that hemodialysis patients have immune compromised systems and infections are more common such as diagnosed and undiagnosed UTIs. It was showed also that hemodialysis patients are more susceptible to UTIs and UTIs are the second cause for hospital admission in patients with chronic kidney diseases[25].

The population study comprised 58% females and 42% males and this was dependent on nature of case and all cases were of random choice according to severity of disease complications associated with renal failure with hemodialysis. The prevalence of infections was increased by increasing age range as 13, 39 and 48% of infections (positive bacterial cultures) were detected in age ranges <40, 40-60 and >60 years, respectively and this is because chronic hemodialysis patients are at high risk for infection due to their immunocompromised nature and because the processes of hemodialysis require vascular access and special care for prolonged periods[26].

In correlation between source of clinical specimens and the physician diagnosis, about 50% of hemolysis patients (100 patients) that were subjected to urine analysis were suffering from diagnosed and undiagnosed UTI and this is could be due to that hemodialysis patients require long term central venous catheters, total parental nutrition and chemotherapy; however, catheters are not exempt from complications of infections[27].

Patients (20 ones) subjected to blood cultures were diagnosed as suffering from bacteremia/septicemia and fever and this is a common problem among hemodialysis patients. Almost catheter's and urinary dialysates cultures were taken from patients infected mostly by either diagnosed or undiagnosed UTIs and this is in conform with many results in this respect[28,29].

The 100 bacterial isolates were distributed as 76% Gram negative bacilli and 24% Gram positive cocci, this result coupled with the findings of Enan *et al.*[11,12] and Chervet *et al.*[5] who showed that almost UTIs causal pathogens are opportunistic bacilli which become infectious in immunocompromised patients.

The bacterial strains identified herein were highly susceptible to imipenem (87%) followed by amikacin (71%) ofloxacin (68%), nitrofurantoin (64%) and ciprofloxacin (62%) and this is in agreement with latter published results[30,31]. On

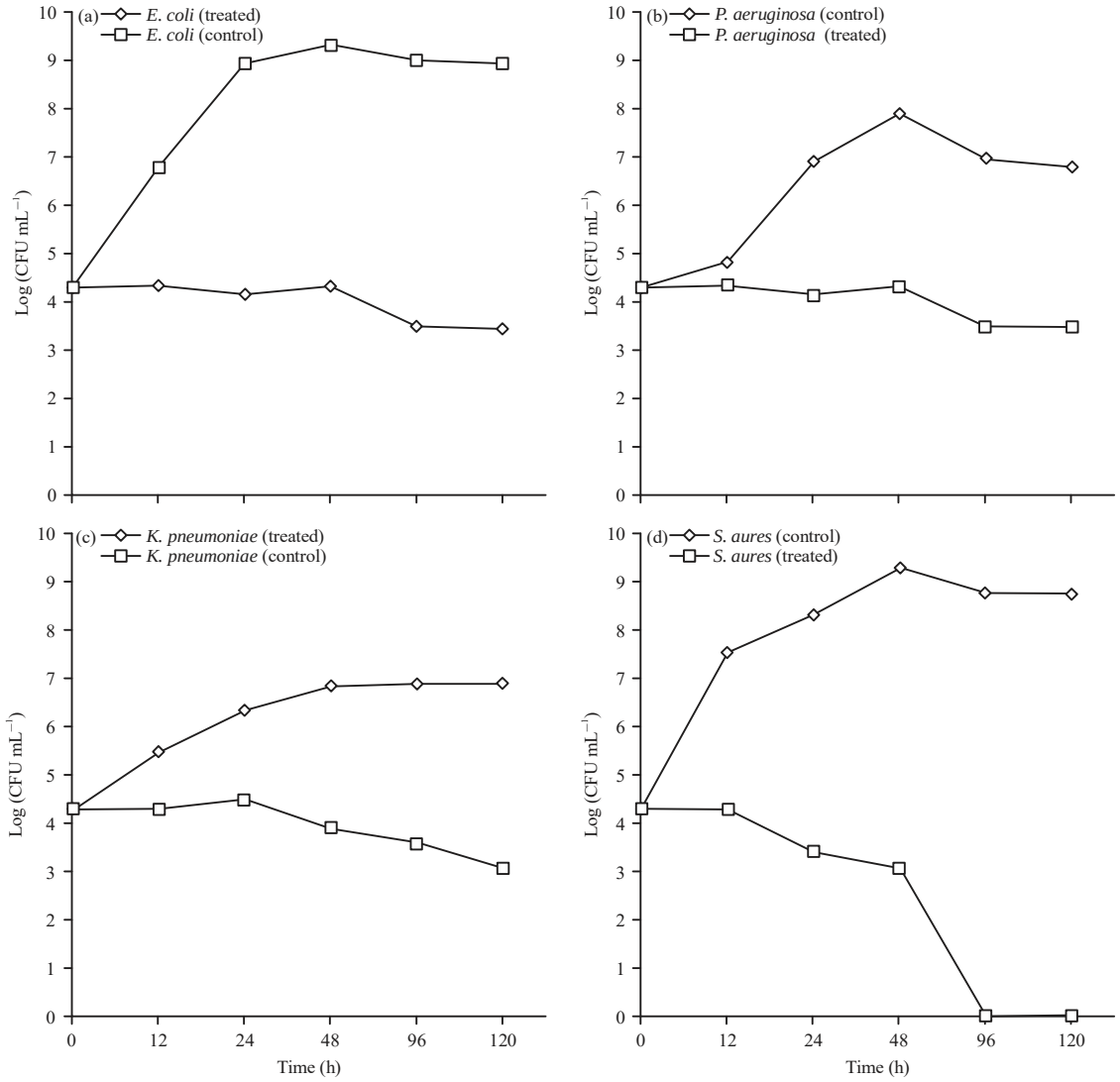


Fig. 4(a-d): Inhibition of (a) *P. aeruginosa* RF22, (b) *E. coli* RF27, (c) *K. pneumoniae* RF51 and (d) *S. aureus* RF55 by CFS of NM_2 *E. faecium* isolated from urine of healthy man

the other hand, 76, 73, 68 and 66% of the 100 bacterial isolates were resistant to cephalothin; sulphomethoxazole. trimethoprim, amoxicillin/clavulinic acid, cefaclor respectively. In view of literature and except for the standard resistance of *S. aureus* to either methicillin (MRSA) or vancomycin (VRSA), there is no standard map of antibiotic resistance phenomena of bacteria; such phenomena are due many reasons such as thickening of cell wall, modification of site receptors, secretion of β-lactamases and genetic reasons[8,9,32].

About 20% of bacterial isolates in this study were MDR isolates and this is in confirm with later published results in this respect[18,29,33]. The 100 bacterial isolates were identified by API-Kits and based on the results obtained, *E. coli* bacteria were the most dominant strains (35 strains) and this is coupled with the findings of this is possible because *E. coli* is a naturally inhabitant opportunistic organism of urogenital system and could be infective in immune compromised patients which are the case herein. Those *E. coli* strains were isolated from urine or urinary dialysates and were involved in both diagnosed and undiagnosed UTIs. In addition, about 7, 3 and 3% of the MDR strains (20 strains) were *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, respectively[19,25,26].

The MDR *P. aeruginosa* strains were isolated form UTIs patients with or without diabetic symptoms and this is coupled with later published results[34] *K. pneumoniae* was also isolated from hemodialysis patients from urine, kidney used dialysate, blood[35]. Finally, the MDR *S. aureus* (3 strains) pathogen were isolated from either blood or urine of

hemodialysis patients; this is because *S. aureus* is an invasive pathogen and frequent cause of skin and soft tissue as well as blood-stream infections[36].

Due to the minor elusive results appeared from biochemical identification the more MDR strains were characterized molecularly by 16S rRNA cataloging analysis which confirmed successful biochemical identification procedures[37].

There is a great challenge to control MDR infections bacteria, in general and that cause infections in hemodialysis patients, in particular, by natural agents. In this regard, *E. faecium* NM_2 was isolated from urine of healthy man and inhibited many pathogenic bacteria from UTIs patients, in such study it was an inversely proportion between probiotic bacteria and UTIs bacteria. This NM_2 strain showed promised probiotic[11,12,38]. This probiotic NM_2 strain inhibited distinctively the MDR bacteria employed herein in this study. Other recent studies showed promising use of probiotics and modified natural proteins in biocontrol of MDR bacterial pathogens[9,17,39-41].

It is highly recommended from this study that the hemodialysis processes must be carried out under completely aseptic conditions. Other treatment protocols using probiotics to bio-control MDR bacteria should be used.

Further work will be needed to study the effect of the probiotic bacterium *E. faecium* NM_2 on pathogenic MDR bacteria *in vivo*. The work in this respect is in progress.

SIGNIFICANCE STATEMENT

The study employed herein discovers that the probiotic bacteria isolated from urine of healthy men could be useful in inhibition of MDR bacteria isolated from hemodialysis patients suffering from disease complications. Molecular characterization of MDR bacteria at hemodialysis patients is necessary to give other scientific knowledge about the nature and epidemiology of bacteria.

REFERENCES

1. Knezevic, V., T. Durdevic-Mirkovic, D. Bozic, G. Majstorovic-Strazmester, I. Mitic and L. Gvozdenovic, 2018. Risk factors for catheter-related infections in patients on hemodialysis. Vojnosanitetski Pregled, 75: 159-166.
2. Dalrymple, L.S., R. Katz, B. Kestenbaum, I.H. de Boer, L. Fried, M.J. Sarnak and M.G. Shlipak, 2012. The risk of infection-related hospitalization with decreased kidney function. Am. J. Kidney Dis., 59: 356-363.
3. Suzuki, M., N. Satoh, M. Nakamura, S. Horita, G. Seki and K. Moriya, 2016. Bacteremia in hemodialysis patients. World J. Nephrol., 5: 489-496.
4. Fram, D., M.F.P. Okuno, M. Taminato, V. Ponzio and S.R. Manfredi *et al*., 2015. Risk factors for bloodstream infection in patients at a Brazilian hemodialysis center: A case-control study. BMC Infect. Dis., Vol. 15. 10.1186/s12879-015-0907-y.
5. Chervet, D., O. Lortholary, J.R. Zahar, A. Dufougeray, B. Pilmis and H. Partouche, 2018. Antimicrobial resistance in community-acquired urinary tract infections in Paris in 2015. Med. Maladies Infect., 48: 188-192.
6. Inyinbor, A.A., O.S. Bello, A.E. Fadiji and H.E. Inyinbor, 2018. Threats from antibiotics: A serious environmental concern. J. Environ. Chem. Eng., 6: 784-793.
7. Abdel-Shafi, S., A. Osman, G. Enan, M. El-Nemer and M. Sitohy, 2016. Antibacterial activity of methylated egg white proteins against pathogenic G^+ and G^- bacteria matching antibiotics. SpringerPlus, Vol. 5. 10.1186/s40064-016-2625-3.
8. Abdel-Shafi, S., A.R. Al-Mohammadi, S. Hamdi, A.H. Moustafa and G. Enan, 2019. Biological characterization and inhibition of *Streptococcus pyogenes* ZUH1 causing chronic cystitis by *Crocus sativus* methanol extract, bee honey alone or in combination with antibiotics: An *in vitro* study. Molecules, Vol. 24. 10.3390/molecules24162903.
9. Abdel-Shafi, S., A.R. Al-Mohammadi, A. Osman, G. Enan, S. Abdel-Hameid and M. Sitohy, 2019. Characterization and antibacterial activity of 7S and 11S globulins isolated from cowpea seed protein. Molecules, Vol. 24, No. 6. 10.3390/molecules24061082.
10. Abdel-Shafi, S., A. Osman, A.R. Al-Mohammadi, G. Enan, N. Kamal and M. Sitohy, 2019. Biochemical, biological characteristics and antibacterial activity of glycoprotein extracted from the epidermal mucus of African catfish (*Clarias gariepinus*). Int. J. Biol. Macromol., 138: 773-780.
11. Enan, G., G. El-Didamony, E.H. Mohamed and A. Zakaria, 2014. Novel antibacterial activity of *Enterococcus faecium* NM_2 Isolated from urine of healthy people. Asian J. Applied Sci., 7: 66-78.
12. Enan, G., A.R. Al-Mohammadi, G. El-Didamony, M.E.F. Abdel-Haliem and A. Zakaria, 2014. Antimicrobial activity of *Enterococcus faecium* NM_2 isolated from urine: Purification, characterization and bactericidal action of enterocin NM_2. Asian J. Applied Sci., 7: 621-634.
13. Enan, G., I.A. Abo-El-Khair, S. Abdel-Shafi and A.R. Al-Mohammadi, 2015. Evaluation of the use of *Enterococcus faecium* NM_2 as a probiotic for inhibition of some urogenital pathogens. J. Food Agric. Environ., 13: 2-7.
14. Abdel-Shafi, S., S.M. Ouda, I. Elbalat and G. Enan, 2013. Characterization and identification of multidrug resistant bacteria from some Egyptian patients. Biotechnology, 12: 65-73.

15. CLSI., 2008. Performance standards for antimicrobial susceptibility testing. 18th Informational Supplement, CLSI Document No. M100-S18, Clinical and Laboratory Standards Institute, Wayne, PA., USA.
16. Holt, J.G., 1994. Facultatively Anaerobic Gram-Negative Rods, Subgroup 1: Family Enterobacteriaceae. In: Bergey's Manual of Determinative Bacteriology, Holt, J.G. (Ed.). 9th Edn., Lippincott Williams and Wilkins, Baltimore, MA., USA., ISBN-13: 9780683006032, pp: 175-189.
17. Enan, G., A.A. El-Essawy, M. Uyttendaele and J. Debevere, 1996. Antibacterial activity of *Lactobacillus plantarum* UG1 isolated from dry sausage: Characterization, production and bactericidal action of plantaricin UG1. Int. J. Food Microbiol., 30: 189-215.
18. Enan, G., S. Abdel-Shafi, M.F. Abdel-Haliem and S. Negm, 2013. Characterization of probiotic lactic acid bacteria to be used as starter and protective cultures for dairy fermentations. Int. J. Probiot. Prebiot., 8: 157-164.
19. Enan, G., S. Abdel-Shafi, S.M. Ouda and I. El-Balat, 2013. Genetic linkage of the antibiotic resistance ability in the *Escherichia coli* UR_4 strain isolated from urine. J. Med. Sci., 13: 261-268.
20. Mathews, D.H., J. Sabina, M. Zuker and D.H. Turner, 1999. Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. J. Mol. Biol., 288: 911-940.
21. Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller and D.J. Lipman, 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucl. Acids Res., 25: 3389-3402.
22. De Man, J.C., M. Rogosa and M.E. Sharpe, 1960. A medium for the cultivation of lactobacilli. J. Applied Bacteriol., 23: 130-135.
23. Ouda, S.M., J. Debevere and G. Enan, 2014. Purification and biochemical characterization of plantaricin UG1: A bacteriocin produced by *Lactobacillus plantarum* UG1 isolated from dry sausage. Life Sci. J., 11: 271-279.
24. Enan, G., S. Hamdy, S. Abdel-Shafi and A.R. Al-Mohammadi, 2016. Biological characteristics and inhibition by both natural agents and antibiotics of *Streptococcus pyogenes*. Res. J. Med. Sci., 10: 573-586.
25. Abdel-Salam, H., T. El-Khamissy, G. Enan and C. Hollenberg, 2001. Expression of mouse anticreatine kinase (MAK33) monoclonal antibody in the yeast *Hansenula polymorpha*. Applied Microbiol. Biotechnol., 56: 157-164.
26. Abdel-Haliem, M.E.F., E. Tartour and G. Enan, 2016. Characterization, production and partial purification of a bacteriocin produced by *Lactobacillus plantarum* LPS10 Isolated from pickled olives. Res. J. Pharmaceut. Biol. Chem. Sci., 7: 2362-2371.
27. Fernandez-Hidalgo, N., B. Almirante, R. Calleja, I. Ruiz and A.M. Planes *et al*., 2006. Antibiotic-lock therapy for long-term intravascular catheter-related bacteraemia: Results of an open, non-comparative study. J. Antimicrob. Chemother., 57: 1172-1180.
28. Richa, C., C.S. Bhushan, S.P. Kumar, P.N. Dev and P. Nabaraj, 2016. Bacteriology of urinary tract infection of chronic renal failure patients undergoing for hemodialysis. J. Microbiol. Exp., Vol. 3, No. 3. 10.15406/jmen.2016.03.00089.
29. Chaudry, M.S., G.H. Gislason, A.L. Kamper, M. Rix and A.R. Larsen *et al*., 2019. Increased risk of *Staphylococcus aureus* bacteremia in hemodialysis-a nationwide study. Hemodial. Int., 23: 230-238.
30. Yang, Q., H. Zhang, Y. Wang, Z. Xu and G. Zhang *et al*., 2017. Antimicrobial susceptibilities of aerobic and facultative gram-negative bacilli isolated from Chinese patients with urinary tract infections between 2010 and 2014. BMC Infect. Dis., Vol. 17. 10.1186/s12879-017-2296-x.
31. Choe, H.S., S.J. Lee, Y.H. Cho, M. Cek and Z. Tandogdu *et al*., 2018. Aspects of urinary tract infections and antimicrobial resistance in hospitalized urology patients in Asia: 10-Year results of the Global Prevalence Study of Infections in Urology (GPIU). J. Infect. Chemother., 24: 278-283.
32. Abdel-Shafi, S., A.R. Al-Mohammadi, S. Negm and G. Enan, 2014. Antibacterial activity of *Lactobacillus delbreukii* subspecies *bulgaricus* isolated from Zabady. Life Sci. J., 11: 264-270.
33. Osman, A., G. El-Didamony, M. Sitohy, M. Khalifa and G. Enan, 2016. Soybean glycinin basic subunit inhibits methicillin resistant-vancomycin intermediate *Staphylococcus aureus* (MRSA-VISA) *in vitro*. Int. J. Applied Res. Nat. Prod., 9: 17-26.
34. Ferreiro, J.L.L., J.A. Otero, L.G. Gonzalez, L.N. Lamazares and A.A. Blanco *et al*., 2017. *Pseudomonas aeruginosa* urinary tract infections in hospitalized patients: Mortality and prognostic factors. PLoS ONE, Vol. 12. 10.1371/journal.pone.0178178.
35. Cristea, O.M., C.S. Avramescu, M. Balasoiu, F.D. Popescu, F. Popescu and M.O. Amzoiu, 2017. Urinary tract infection with *Klebsiella pneumoniae* in patients with chronic kidney disease. Curr. Health Sci. J., 43: 137-148.
36. Thomer, L., O. Schneewind and D. Missiakas, 2016. Pathogenesis of *Staphylococcus aureus* bloodstream infections. Annu. Rev. Pathol.: Mech. Dis., 11: 343-364.
37. Cohen, S.H., D.N. Gerding, S. Johnson, C.P. Kelly and V.G. Loo *et al*., 2010. Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). Infect. Control Hosp. Epidemiol., 31: 431-455.

38. Enan, G., M.E.F. Abdel-Haliem and M. Tartour, 2014. Evaluation of the antimicrobial activity, starter capability and technological properties of some probiotic bacteria isolated from Egyptian pickles. Life Sci. J., 11: 976-985.
39. El-Sayed, T.I., D. Atef, M. Amer, A. Mahdy and G. Enan, 2015. Molecular characterization and inhibition by natural agents of multidrug resistant Candida strains causing vaginal candidiasis. Res. J. Med. Sci., 9: 1-7.
40. Enan, G., S. Abdel-Shafi, S. Ouba and S. Negm, 2013. Novel antibacterial activity of *Lactococcus lactis* subspecies *Lactis* Z_{11} isolated from Zabady. Int. J. Biomed. Sci., 9: 174-180.
41. Sahar-Eissa, A., A.S. Saad, G. Enan and K.A. El-Dougdoug, 2016. Evaluation the using of potential probiotic antibacterial against urogental tract infection *in-vitro*. Res. J. Pharmaceut. Biol. Chem. Sci., 7: 976-983.