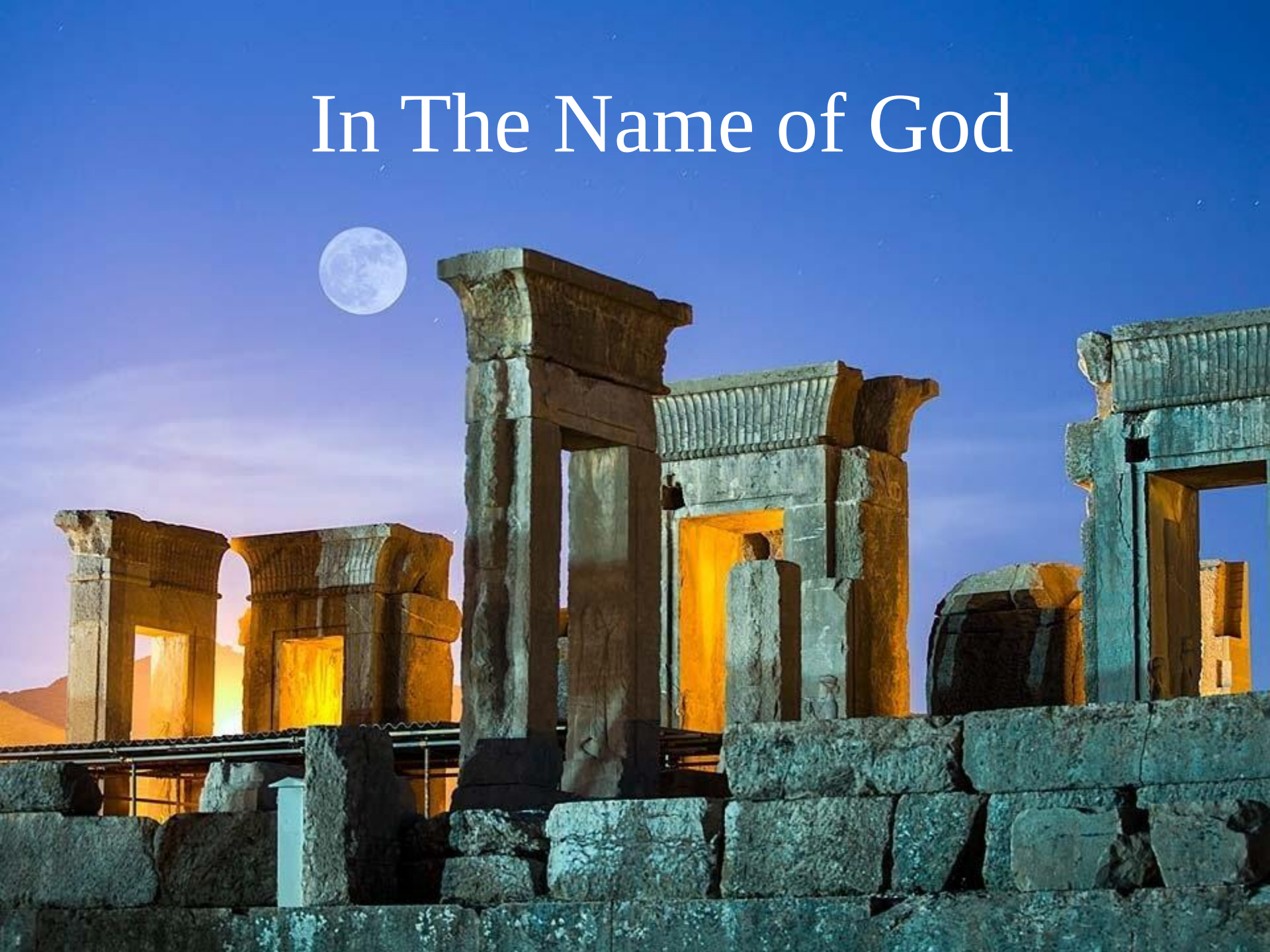
In The Name of God



Fecal carriage of carbapenem resistance Enterobacteriaceae among inpatients in university hospital in Iran

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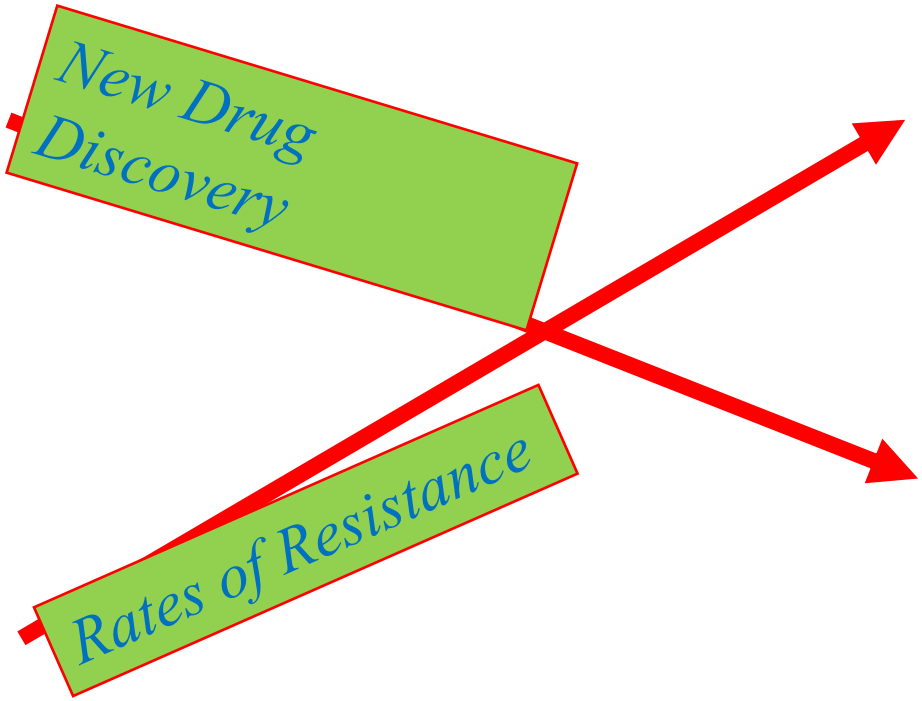
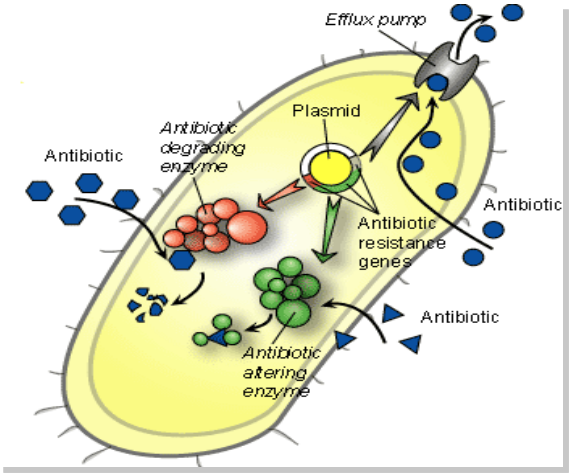
Antibiotic resistance is a global problem, but the solutions are at the national and regional level.

The benefits of conservation efforts accrue locally while contributing to antibiotic effectiveness at the global scale.

We Have a Basic Problem





World Health Day – 7 April 2011

Antimicrobial resistance: no action today, no cure tomorrow

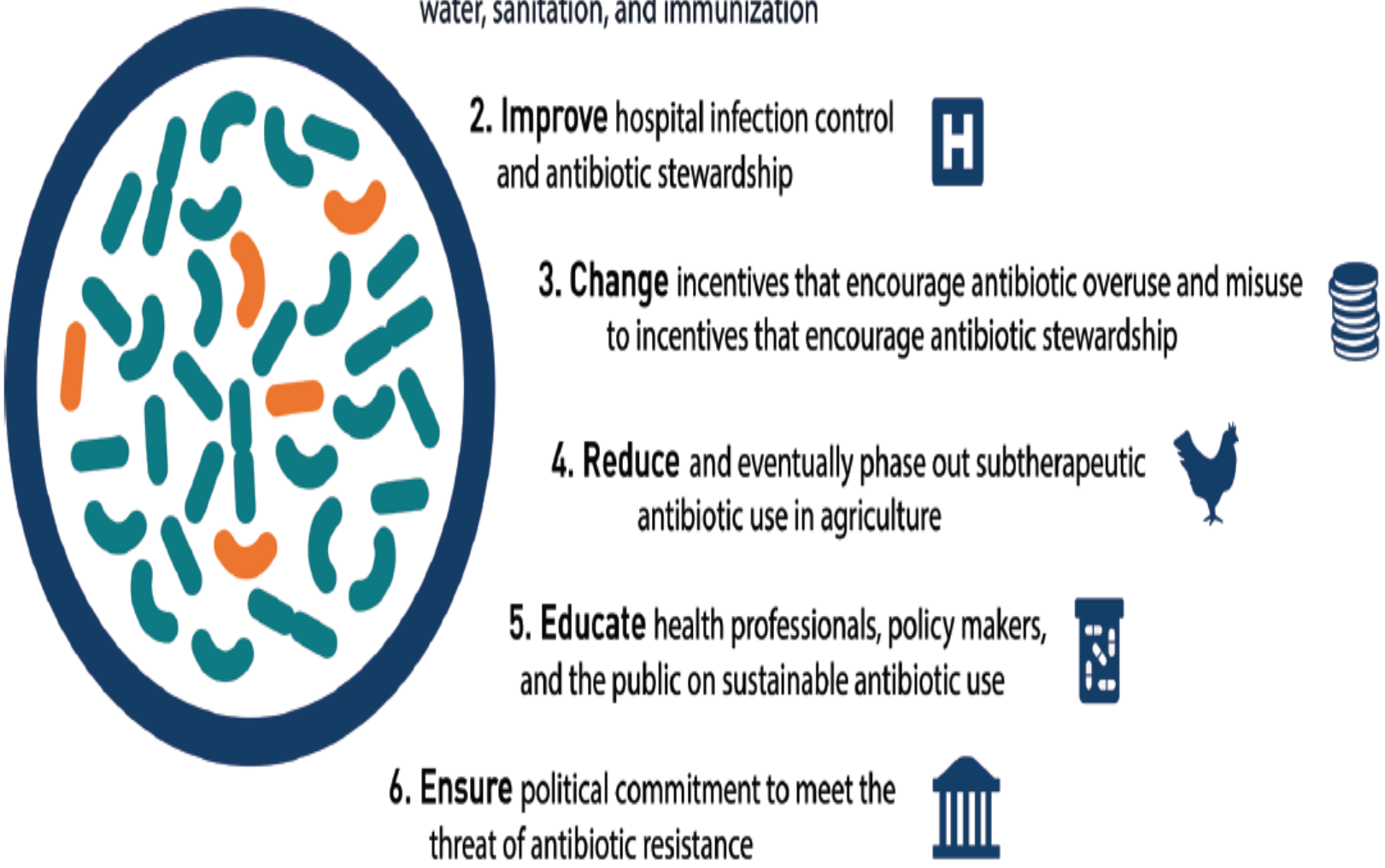


FIGURE 5-1: Six strategies needed in national antibiotic policies

WHO priority pathogens list for R&D of new antibiotics (2017)

❑ **Priority 1: CRITICAL**

- Carbapenem-resistant *Enterobacteriaceae*
- *Acinetobacter baumannii*, carbapenem-resistant
- *Pseudomonas aeruginosa*, carbapenem-resistant

❑ **Priority 2: HIGH**

- *Enterococcus faecium*, vancomycin-resistant
- *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant
- *Helicobacter pylori*, clarithromycin-resistant
- *Campylobacter* spp., fluoroquinolone-resistant
- *Salmonellae*, fluoroquinolone-resistant
- *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant

❑ **Priority 3: MEDIUM**

- *Streptococcus pneumoniae*, penicillin-non-susceptible
- *Haemophilus influenzae*, ampicillin-resistant
- *Shigella* spp., fluoroquinolone-resistant

World Health Organization

Our projects:

- Evaluation of phenotypic and molecular epidemiology of carbapenemase producing Enterobacteriaceae isolated from carriers and clinical samples.

- Study of phenotypic, genotypic characteristics and molecular epidemiology of predominant isolates of ESBL producing Enterobacteriaceae family (EPE) isolated from carriers and clinical samples.

Our projects:

- Molecular typing of carbapnemase producing Enterobacteriaceae isolates and determine the genomic structure of plasmids carrying carbapenemase genes.

Our projects:

- The aim of this study was to evaluate the prevalence and risk factors associated with CRE fecal colonization among inpatients.

Two main approaches to prevent the spread of CRE

- Detection of infected patients
- Detection of carriers

How does a CRE patient present?

- Infection versus Colonization
- A patient with CRE can be colonized and/or infected.

Why detecting rectal colonized patients with CPE is so important?

- Fecal carriage with CPE is a marker for infection with CPE
- - nearly 10% of patients with positive CPE rectal carriage are latter positive in a clinical sample (85% being true infection) - 11 days median time interval (range 3-27 days) between positive rectal screening and positive clinical specimen
- **prevent transmission** of the resistant bacteria in community and hospital
- **prevent** the development of **nosocomial** outbreaks due to the multidrug-resistant bacteria
- Screening test for surveillance program

The human gut microbiome as a transporter of antibiotic resistance genes between continents

Johan Bengtsson-Palme[a], Martin Angelin[b], Mikael Huss[c], Sanela Kjellqvist[c], Erik Kristiansson[d], Helena Palmgren[b], D.G. Joakim Larsson[a] and Anders Johansson[#,e]

+ Author Affiliations

ABSTRACT

Previous studies of antibiotic resistance dissemination by travel have, by targeting only a select number of cultivable bacterial species, omitted most of the human microbiome. Here, we used explorative shotgun metagenomic sequencing to address the abundance of >300 antibiotic resistance genes in fecal specimens from 35 Swedish students taken before and after exchange programs on the Indian peninsula or in central Africa. All specimens were additionally cultured for extended-spectrum beta-lactamase (ESBL) producing enterobacteria and the isolates obtained genome sequenced. The overall taxonomic diversity and composition of the gut microbiome remained stable comparing before and after travel, but with increasing abundance of Proteobacteria in 25/35 students. The relative abundance of antibiotic resistance genes increased, most prominently for genes encoding resistance to sulfonamide (2.6-fold increase), trimethoprim (7.7-fold) and beta-lactams (2.6-fold). Importantly, the increase observed occurred without any antibiotic intake. Of 18 students visiting the Indian peninsula, 12

This Article

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What specimens?

- stools and rectal swabs are the most suitable specimens for performing this screening process.

- It is imperative to point out that the screening process on admission still requires the patients to be kept in strict isolation prior to results being obtained (at least for 48 h).

Who must be screened?

- Screening should include at least **‘at-risk’** patients, such as those in **intensive care units**, and transplantation and immunocompromised patients.
- If a patient is confirmed as being infected or colonized by a carbapenemase producer, the screening program should be extended to **neighboring patients** on the hospital ward.
- Screening shall be done at least to patients transferred from a **foreign hospital** on addition to any hospital.

Does everyone who gets the bug become sick?

- **No**, some people are colonized.
- Patients with unrecognized colonization with CPE have served as reservoirs for transmission during outbreaks.

Screening of Carriers

- The prevention of spread of carbapenemase producers relies on **early** and **accurate** detection of carriers in hospital units or on admission/discharge either to the hospital or to a specific unit.

Why Enterobacteriaceae?

- Source of community- and hospital-acquired infections.
- Normal gut flora
- They have the propensity to spread easily between humans (hand carriage, contaminated food and water, medical equipment)
- Acquire genetic material through horizontal gene transfer, mediated mostly by plasmids and transposons.

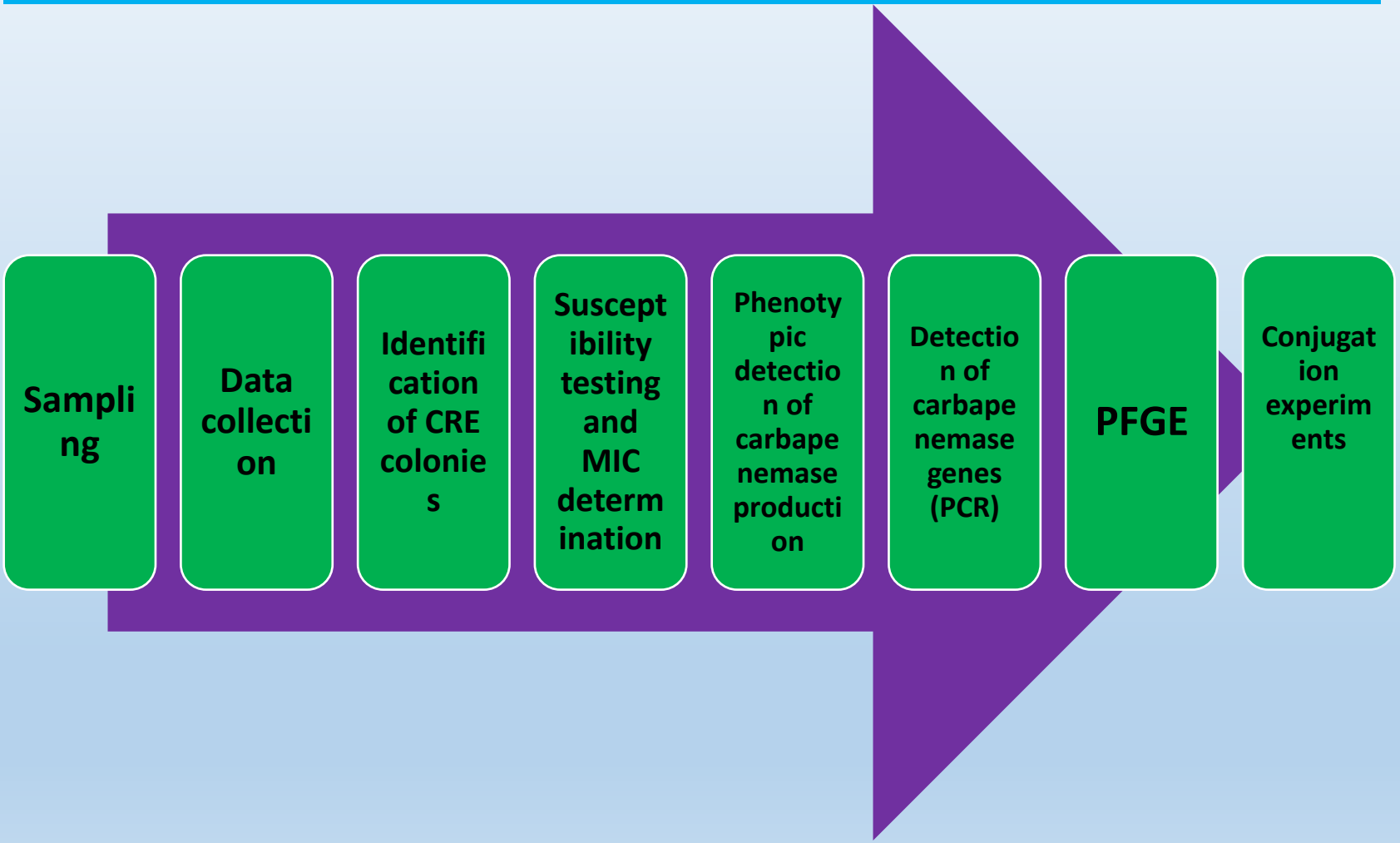
Why Carbapenemases:

- Carbapenemases are a source of concern because :
  - ✓ **They confer resistance to essentially all β-lactams**
  - ✓ **Strains producing carbapenemases often possess resistance mechanisms to a wide-range of antimicrobial agents**
- Carbapenemases **increasingly** have been reported in Enterobacteriaceae in the past 10 years
- Infections with **CPE** are associated with **high mortality rates**
- **No vaccines** are readily available for preventing infections with carbapenemase producers

Carbapenemases: the triple difficulty

- **Difficulty of detection** in the clinic and in the laboratory
- **Difficulty of treatment** (owing to their MDR, XDR, PDR character)
- **Difficulty to limit transmission and spread and to control** outbreak (local, regional, national, pandemic)

Methods


Sampling
Data collection
Identification of CRE colonies
Susceptibility testing and MIC determination
Phenotypic detection of carbapenemase production
Detection of carbapenemase genes (PCR)
PFGE
Conjugation experiments

Sampling

- Hospital based cross-sectional study
- July to November 2015
- Rectal swab specimens were collected from 95 inpatients
- Informed consent was obtained from all participants
- Swab was inserted 2 to 3 cm into the rectum
- Specimens were inoculated immediately in TSB (5mL) containing a 10 μg ETP disk

Data collection

- **Clinical and epidemiologic data were collected from all inpatients**
- **The following data were recorded:**

✓ Age

✓ Sex

✓ Unit of hospitalization

✓ Invasive medical device utilization

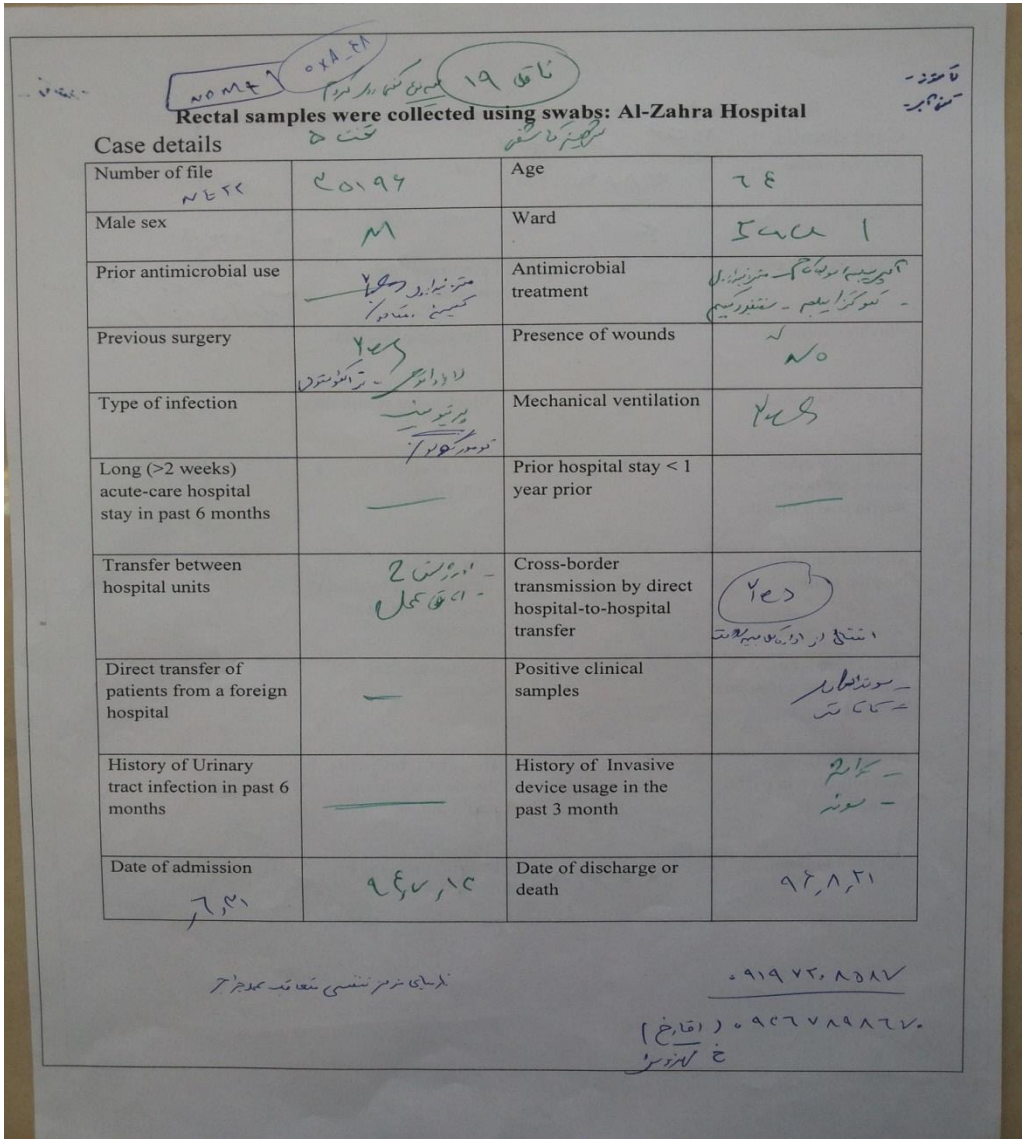
✓ History of surgery, presence of wounds

✓ Transfer from another hospital

✓ Transfer between hospital units

✓ Exposure to antibiotics

Data Collection



Rectal samples were collected using swabs: Al-Zahra Hospital

Case details

Number of file	۲۵۱۹۶	Age	۲۴
Male sex	M	Ward	ICU 1
Prior antimicrobial use		Antimicrobial treatment	
Previous surgery		Presence of wounds	No
Type of infection		Mechanical ventilation	Yes
Long (>2 weeks) acute-care hospital stay in past 6 months	—	Prior hospital stay < 1 year prior	—
Transfer between hospital units		Cross-border transmission by direct hospital-to-hospital transfer	Yes
Direct transfer of patients from a foreign hospital	—	Positive clinical samples	
History of Urinary tract infection in past 6 months	—	History of Invasive device usage in the past 3 month	
Date of admission	۹۴٫۷٫۱۶	Date of discharge or death	۹۴٫۸٫۲۱

CRE screening

- We used two different phenotypic methods for detection of CRE in rectal swab specimens
- Method 1 , MacConkey agar (CDC)
- Method 2, CHROMagar KPC medium
- Isolates were confirmed by using standard biochemical tests and API 20E

CRE screening in rectal swab specimens

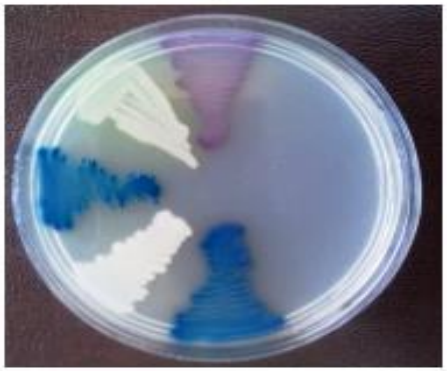
MacConkey agar+ MEM

Sample

Select
TSB + carbapenem disk

Differentiate

CHROMagar KPC



Susceptibility testing and MIC determination (CLSI)

- Disk diffusion method on Muller-Hinton agar plates

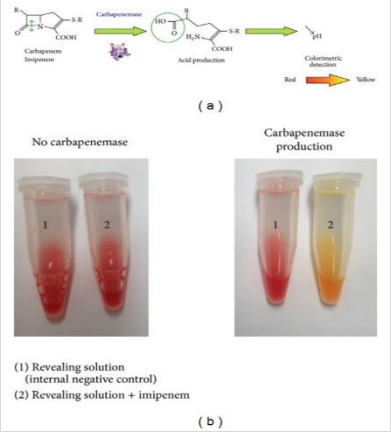
✓ Imipenem, Meropenem, Ertapenem, ceftazidime, cefotaxime, cefepime, ciprofloxacin, amikacin, gentamicin, aztreonam and tigecycline (MAST)

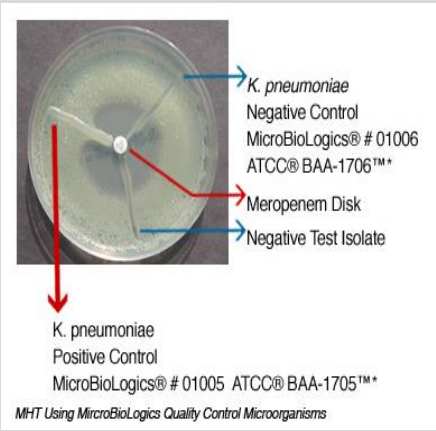
- Minimal inhibitory concentrations (MICs) of IMP, MEM and ETP were determined by gradient test strips

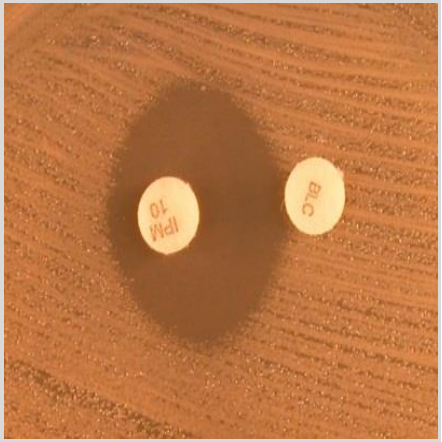
✓ *E. coli* ATCC 25922 was used as control strains

Phenotypic detection of carbapenemase production

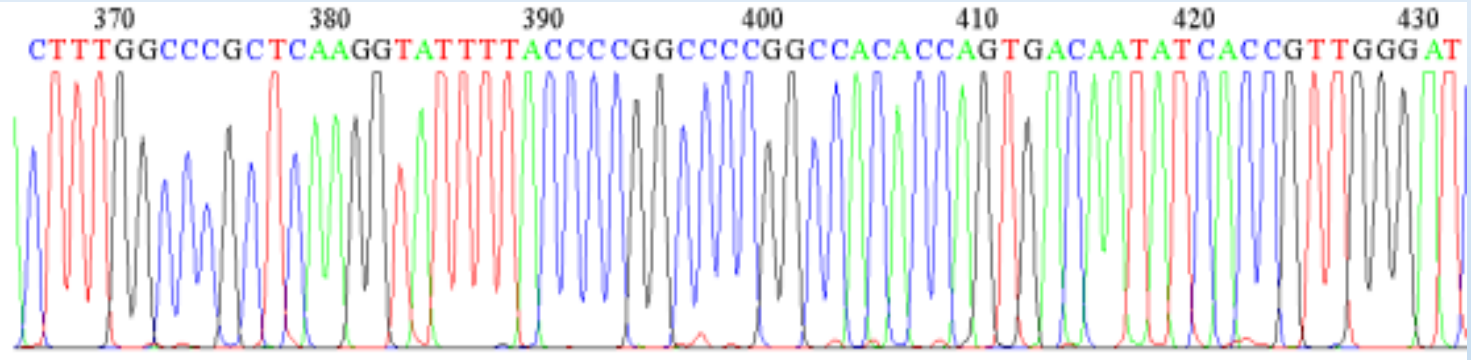
- ❑ Modified Hodge test (MHT)
- ❑ Combined disk test (CDT)
- ✓EDTA ⟹ MBLs
- ❑ MBL-E-test
- ❑ Carba NP





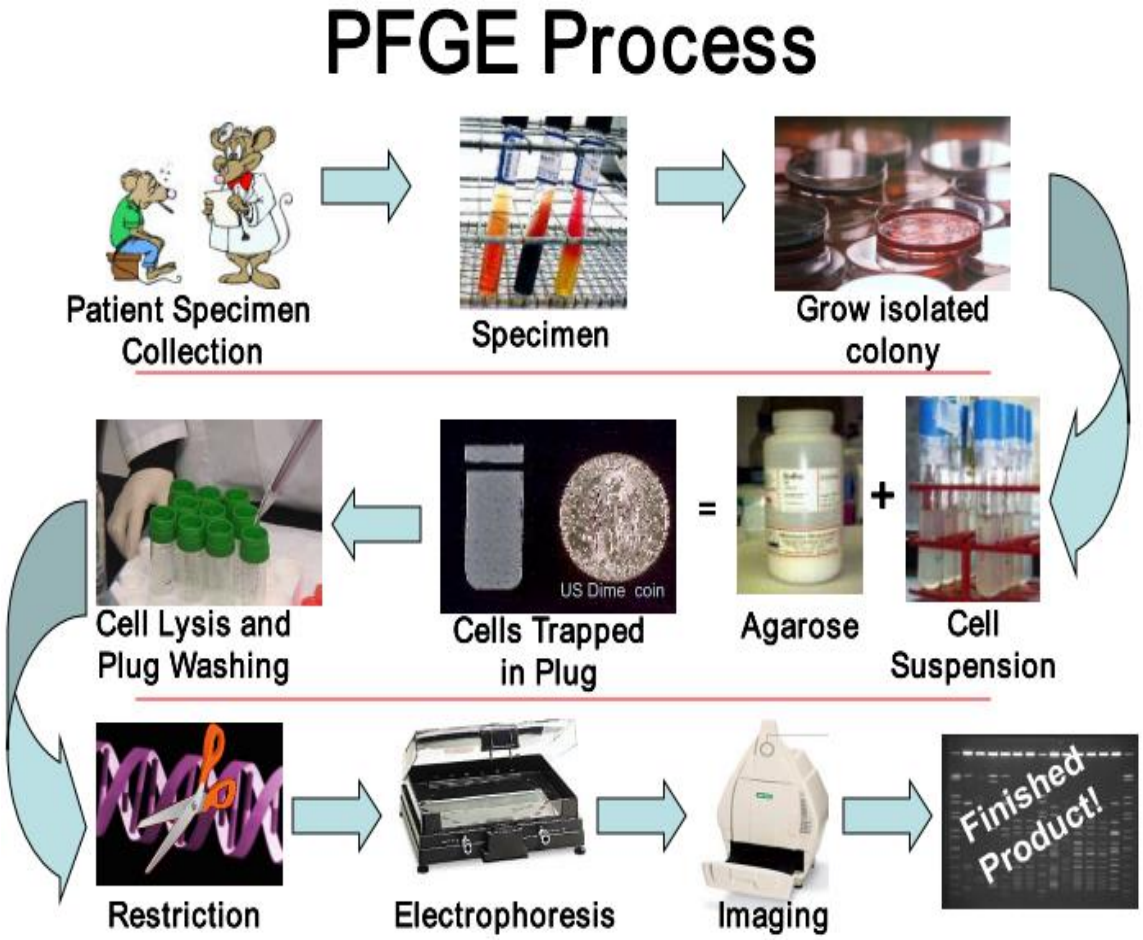


Nucleotide sequences and GenBank accession numbers



- KX467530, KX467529 and KX671151

Pulsed-field gel electrophoresis (PFGE)



- ***K.pneumoniae***
- ***E.coli***
- ***S. marcescens***
- ***E. cloacae***

MLST

✓***K. pneumoniae***

- *gap*A, *inf*B, *mdh*, *pgi*, *pho*E, *rpo*B and *ton*B

❖http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html

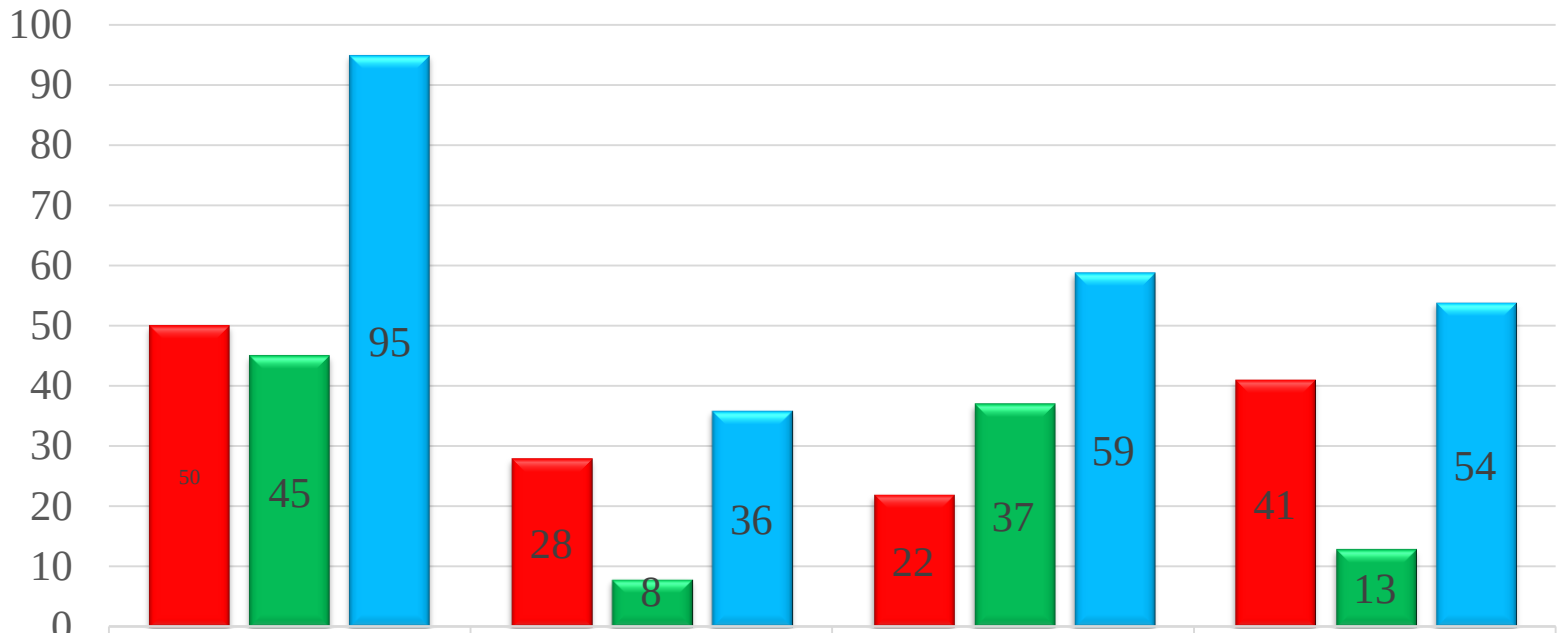
✓***E. coli***

- *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*

❖http://mlst.warwick.ac.uk/mlst/dbs/Ecoli

Results

Rate of colonization of inpatients by CRE in two Al-Zahra and Loghman hospitals



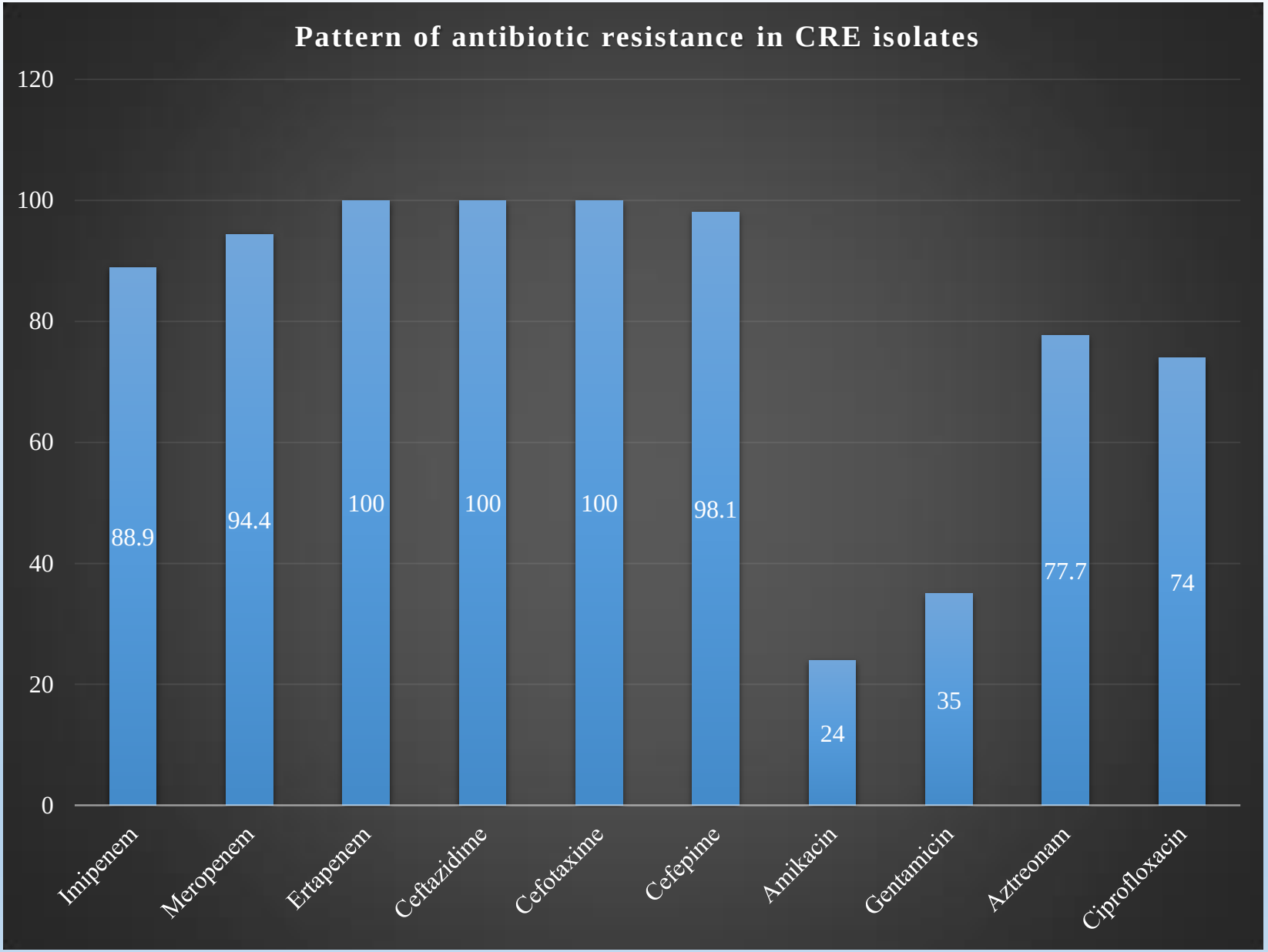
	All patients	Inpatients CRE colonized	Inpatients not CRE colonized	CRE isolates
Hospital A	50	28	22	41
Hospital B	45	8	37	13
Total	95	36	59	54

Hospital A Hospital B Total

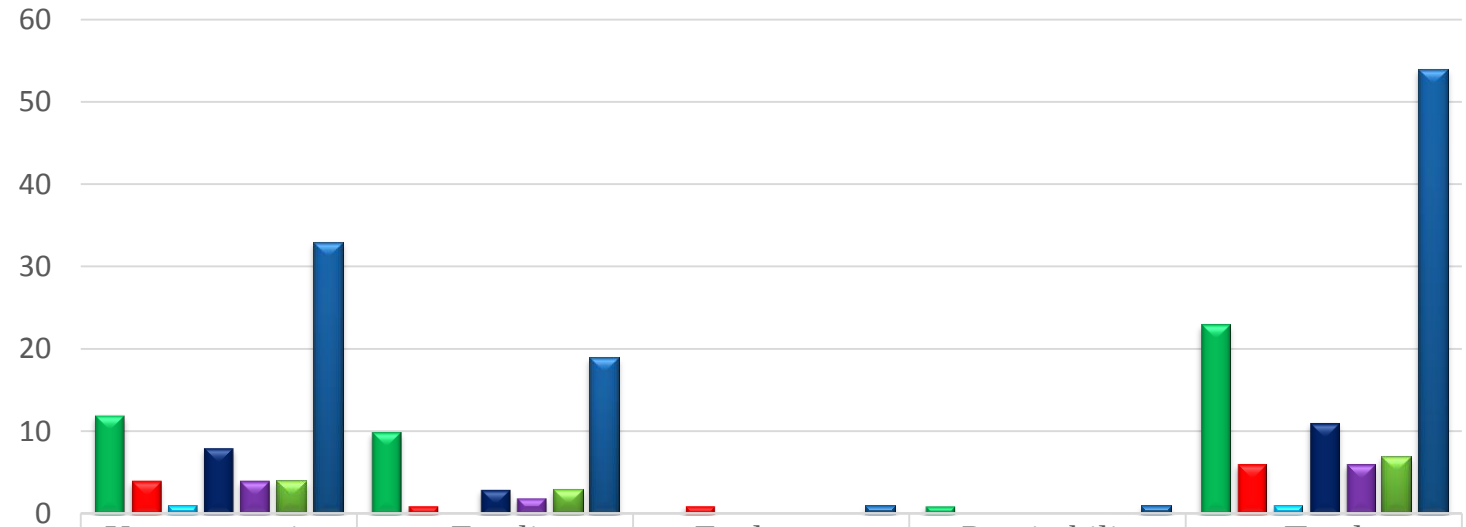
The isolates recovered from 95 rectal samples using 2 different methods

CRE isolates	CHROMagar KPC		MacConkey agar	
	Total	Carbapenemase producing	Total	Carbapenemase producing
K. pneumoniae	33	29	33	29
E.coli	19	16	18	15
E.cloacae	1	1	1	1
P.mirabilis	1	1	-	-
Total isolates	54	47	52	45

- rate of carriage was high (37.9%)
- A higher proportion (28/36; 77.8%) of colonization with CRE isolates was identified among admitted patients in the hospital A compared to hospital B (8 colonized patients)
- CRE multiple colonization → 15 patients


Pattern of antibiotic resistance in CRE isolates
120
100
80
60
40
20
0
88.9
94.4
100
100
100
98.1
24
35
77.7
74
Imipenem
Meropenem
Ertapenem
Ceftazidime
Cefotaxime
Cefepime
Amikacin
Gentamicin
Aztreonam
Ciprofloxacin

Distribution of carbapenemase genes in CRE isolates recovered from carriers in both hospitals.



	K. pneumoniae	E.coli	E. cloacae	P. mirabilis	Total
OXA-48	12	10	0	1	23
NDM-1	4	1	1	0	6
NDM-7	1	0	0	0	1
NDM-1/OXA-48	8	3	0	0	11
NDM-7/OXA-48	4	2	0	0	6
Negative	4	3	0	0	7
Total	33	19	1	1	54

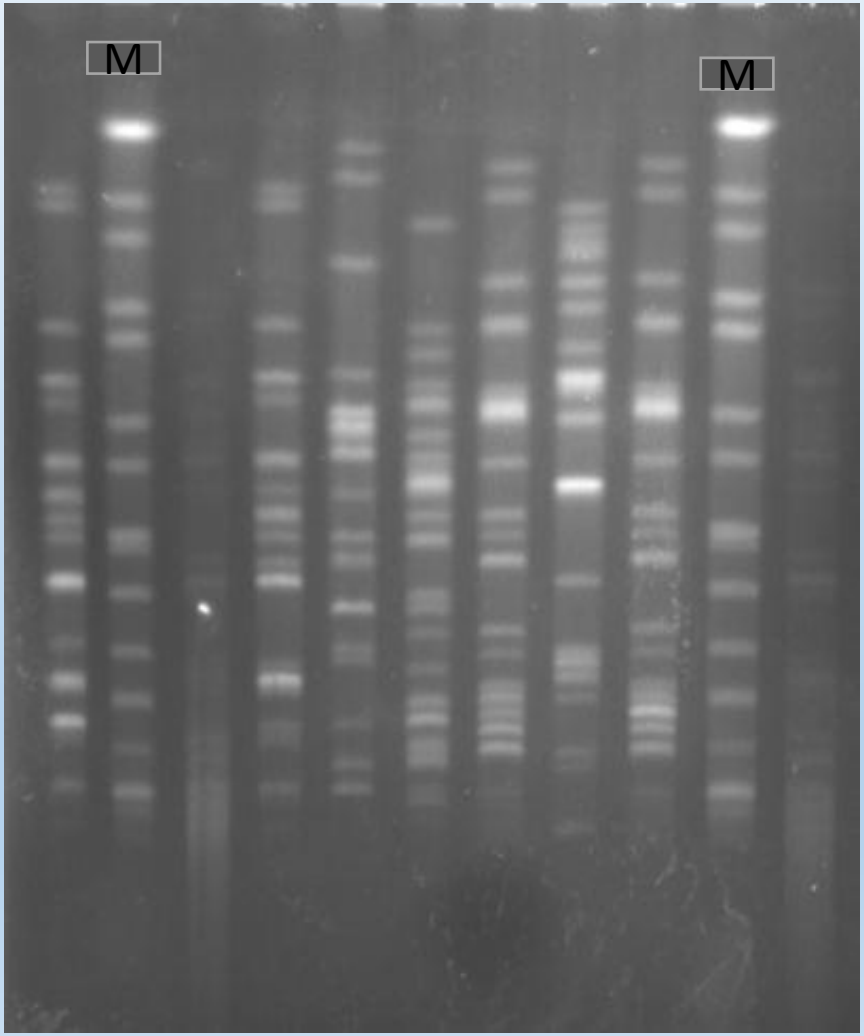
OXA-48 NDM-1 NDM-7 NDM-1/OXA-48 NDM-7/OXA-48 Negative Total

Characteristics of the 54 CRE isolated from intestinal carriage

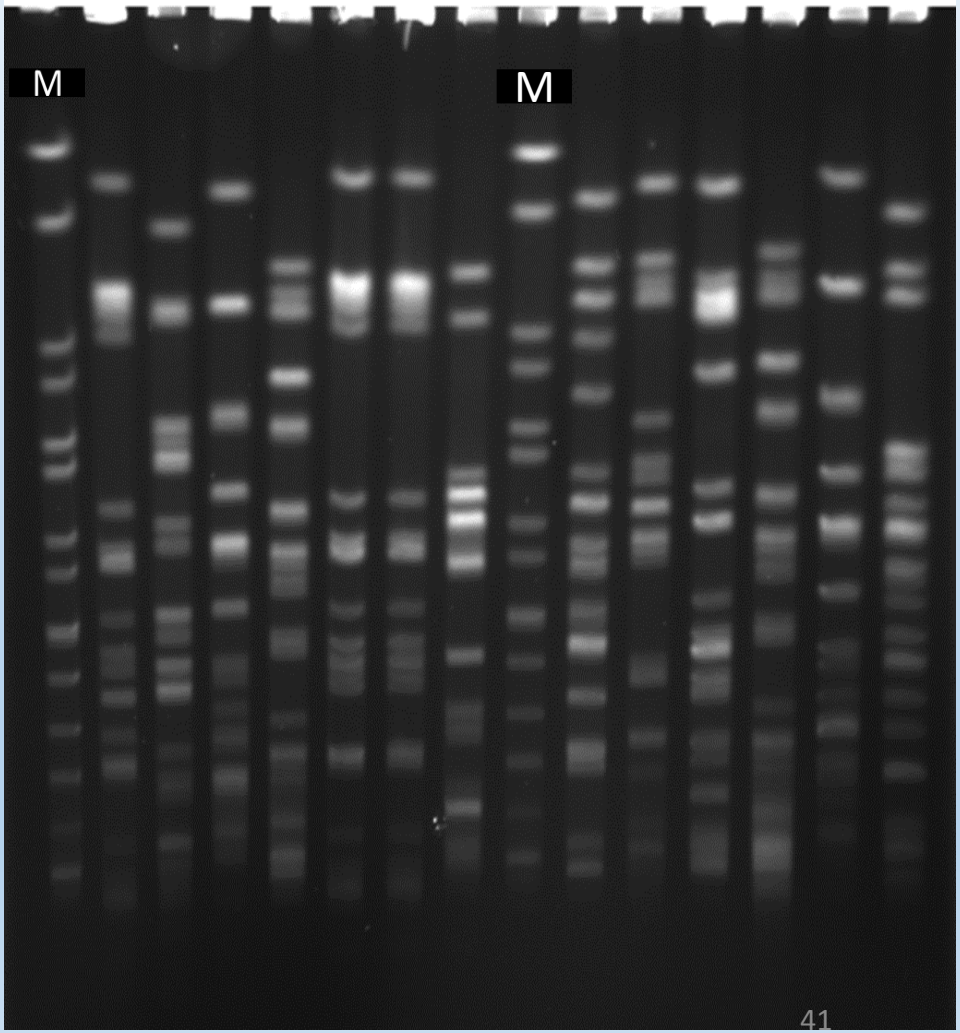
Patient	Species	Ward/Hospital	Data of isolation (day/month)	MHT	Etest-MBL	CDT		MICs (µg/ml)			Carbapenemases	PFGE clasters
						MRP/BO	MRP/EDTA	ETP	MRP	IMP		
P1	K. pneumoniae	ICU-2/ HA	12/10/2015	+	+	-	+	8	8	12	NDM-7, OXA-48	Cluster-I
P2	K. pneumoniae	ICU-2/ HA	12/10/2015	+	+	-	+	8	8	12	NDM-1	Cluster-III
P3	K. pneumoniae	ICU-2/ HA	12/10/2015	+	+	-	+	8	8	16	NDM-7, OXA-48	Cluster-I
	E.coli	ICU-2/ HA	12/10/2015	+	+	-	+	8	8	8	NDM-7, OXA-48	singleton
P4	K. pneumoniae	ICU-2/ HA	12/10/2015	+	+	-	+	8	8	12	NDM-7, OXA-48	-
P5	K. pneumoniae	ICU-2/ HA	12/10/2015	-	+	-	+	8	8	64	NDM-7	singleton
	E.coli	ICU-2/ HA	12/10/2015	+	+	-	+	1	0.5	<4	OXA-48	singleton
P6	K. pneumoniae	ICU-2/ HA	12/10/2015	+	+	-	+	8	1.5	<4	OXA-48	Cluster-I
P7	E.coli	ICU-2/ HA	12/10/2015	+	-	-	-	ND	ND	ND	-	-
P8	K. pneumoniae	ICU-2/ HA	12/10/2015	+	ND	+	+	ND	ND	ND	-	-
P9	K. pneumoniae	ICU-2/ HA	12/10/2015	+	ND	+	+	ND	ND	ND	-	-
	E.coli	ICU-2/ HA	12/10/2015	+	+	-	+	8	8	12	NDM-1, OXA-48	singleton
P10	K. pneumoniae	ICU-2/ HA	15/10/2015	+	+	+	+	8	8	8	OXA-48	Cluster-I
P11	E.coli	ICU-2/ HA	15/10/2015	+	+	-	+	8	8	12	NDM-7, OXA-48	Cluster-I
P12	K. pneumoniae	ICU-2/ HA	15/10/2015	+	-	+	-	8	8	8	OXA-48	Cluster-I
	K. pneumoniae	ICU-2/ HA	15/10/2015	+	+	-	+	8	8	8	OXA-48	Cluster-I
P13	*K. pneumoniae*	ICU-2/ HA	12/10/2015	+	+	-	+	8	8	256	NDM-1, OXA-48	Cluster-IV
	E.coli	ICU-2/ HA	15/10/2015	+	-	-	-	1	0.25	<4	OXA-48	singleton
P14	*K. pneumoniae*	ICU-2/ HA	15/10/2015	+	+	-	+	8	8	8	OXA-48	Cluster-I
	E.coli	ICU-2/ HA	15/10/2015	+	+	-	+	8	8	32	OXA-48	Cluster-II
P15	*K. pneumoniae*	ICU-1/ HA	17/11/2015	+	+	+	+	8	8	16	NDM-1	Cluster-III
P16	*K. pneumoniae*	ICU-1/ HA	17/11/2015	+	+	-	+	8	8	12	NDM-7, OXA-48	singleton
P17	*K. pneumoniae*	ICU-1/ HA	17/11/2015	+	+	-	+	8	8	256	NDM-1, OXA-48	Cluster-I

P18	*K. pneumoniae*	ICU-1/ HA	17/11/2015	+	+	-	-	8	8	12	NDM-1, OXA-48	singleton
	E.coli	ICU-1/ HA	17/11/2015	+	+	-	-	1	0.25	<4	OXA-48	singleton
P19	*E.coli*	ICU-1/ HA	17/11/2015	+	+	-	+	8	8	12	NDM-1, OXA-48	-
P20	*K. pneumoniae*	ICU-1/ HA	17/11/2015	+	+	-	+	8	8	8	OXA-48	Cluster-I
P21	*K. pneumoniae*	ICU-3/ HA	17/11/2015	+	+	-	+	8	8	12	NDM-1, OXA-48	Cluster-III
	E.coli	ICU-3/ HA	17/11/2015	+	+	-	-	8	8	16	NDM-1, OXA-48	singleton
P22	*K. pneumoniae*	ICU-2/ HA	17/11/2015	+	+	-	+	8	8	256	NDM-1, OXA-48	Cluster-IV
P23	*E.coli*	ICU-3/ HA	17/11/2015	+	+	-	+	8	8	16	OXA-48	Cluster-II
P24	*K. pneumoniae*	ICU-3/ HA	17/11/2015	+	+	+	-	8	8	8	NDM-1, OXA-48	Cluster-II
P25	*K. pneumoniae*	ICU-3/ HA	17/11/2015	+	+	-	+	8	8	8	OXA-48	Cluster-I
P26	*K. pneumoniae*	ICU-3/ HA	17/11/2015	+	+	-	+	8	8	8	OXA-48	Cluster-I
	E.coli	ICU-3/ HA	17/11/2015	+	-	-	-	0.25	0.25	<4	OXA-48	singleton
	P.mirabilis	ICU-3/ HA	17/11/2015	+	+	-	+	8	8	48	OXA-48	-
P27	*K. pneumoniae*	ICU-2/ HA	17/11/2015	+	+	+	-	8	2	<4	OXA-48	singleton
	K. pneumoniae	ICU-2/ HA	17/11/2015	+	+	+	-	8	8	6	OXA-48	-
	E.coli	ICU-2/ HA	17/11/2015	+	+	-	-	8	1.5	<4	OXA-48	singleton
P28	*E.coli*	ICU-2/ HA	17/11/2015	+	+	+	-	8	8	6	OXA-48	Cluster-II
	E.coli	ICU-2/ HA	17/11/2015	+	+	-	+	8	8	6	OXA-48	singleton
P29	*K. pneumoniae*	ID/HB	1/7/2015	+	+	-	+	8	8	16	NDM-1, OXA-48	singleton
P30	*E.coli*	ID/ HB	1/7/2015	-	ND	+	-	ND	ND	ND	-	-
P31	*K. pneumoniae*	G- ICU/ HB	18/6/2015	-	ND	+	-	ND	ND	ND	-	-
	K. pneumoniae	G- ICU/ HB	18/6/2015	-	ND	+	-	ND	ND	ND	-	-
P32	*E.coli*	E- ICU/ HB	31/7/2015	-	ND	+	-	ND	ND	ND	-	-
P33	*K. pneumoniae*	G- ICU/ HB	1/8/2015	-	+	-	+	8	8	8	NDM-1	singleton
	E.coli	G- ICU/ HB	1/8/2015	+	+	-	+	8	8	8	NDM-1	singleton
	E.cloacae	G- ICU/ HB	1/8/2015	+	+	-	+	8	3	4	NDM-1	-
P34	*K. pneumoniae*	G- ICU/ HB	20/9/2015	+	+	-	+	8	8	16	NDM-1, OXA-48	Cluster-II
	E.coli	G- ICU/ HB	20/9/2015	+	+	+	-	8	1.5	<4	OXA-48	singleton
P35	*K. pneumoniae*	ID/ HB	18/9/2015	+	-	+	-	8	8	6	OXA-48	singleton
P36	*K. pneumoniae*	E- ICU/ HB	19/10/2015	+	-	+	-	8	8	<4	OXA-48	Cluster-IV
	K. pneumoniae	E- ICU/ HB	19/10/2015	-	+	-	+	8	8	256	NDM-1	singleton

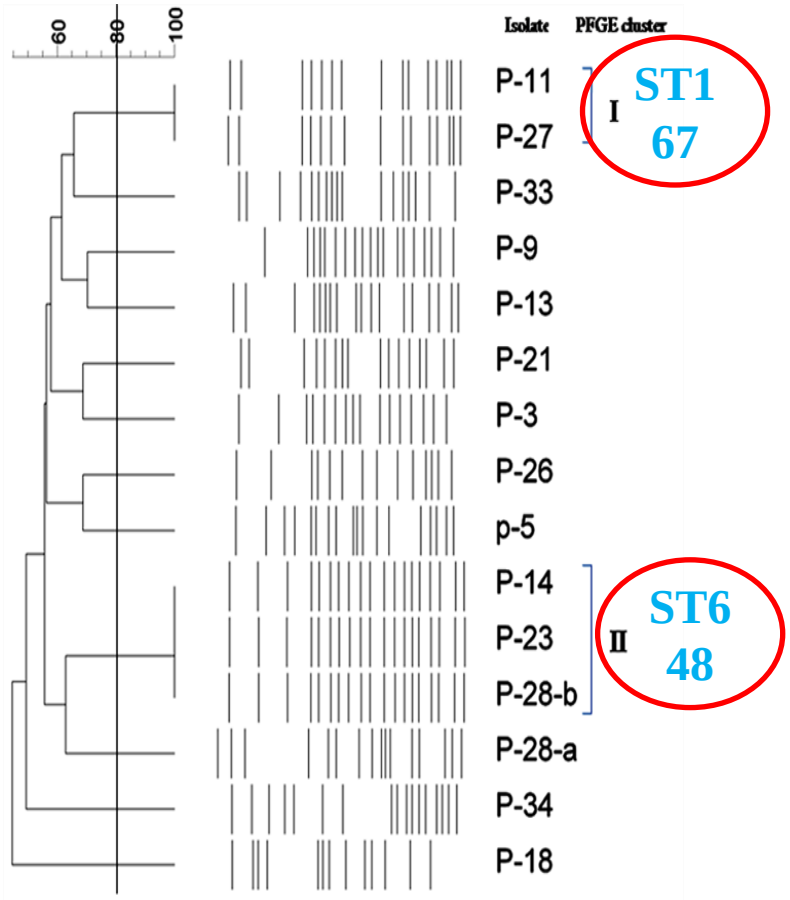
PFGE *E.coli*

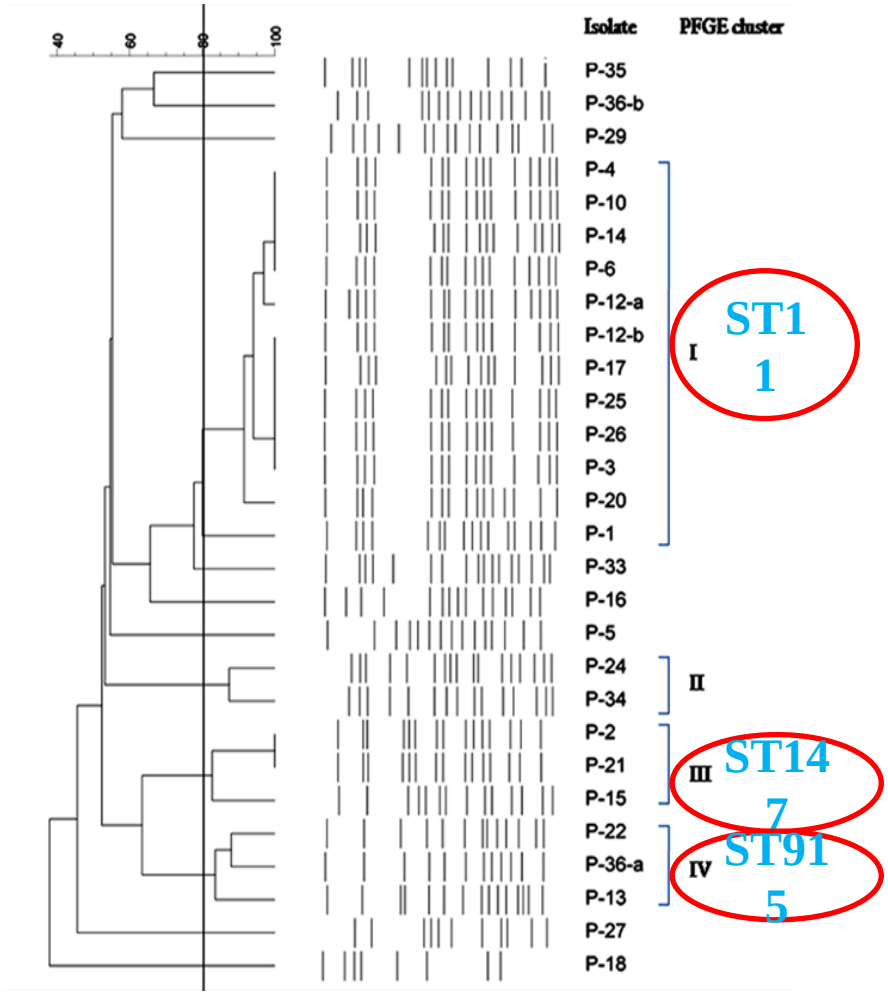


PFGE *K. pneumoniae*



The clonal relatedness among *E.coli* and *K. pneumoniae* isolates recoverd from carriages





- PFGE dendrogram in 15 CP *E.coli*

- PFGE dendrogram in 28 CP *K. pneumoniae*

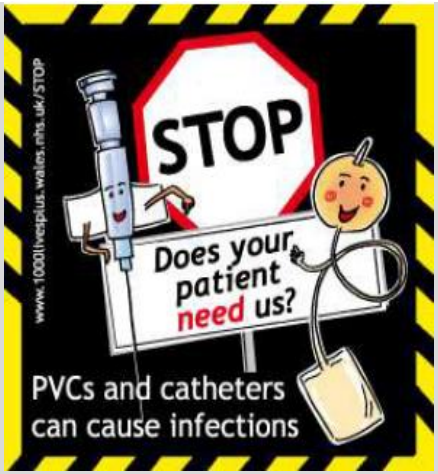
Discussion

Risk Factors for CRE colonization

- ❖ Admission to ICU
  - ➢ mainly ICU-2 (p=0.00004) and general ICU (p=0.007)
- ❖ Surgery (p=0.03)
- ❖ Ventilation (p=0.0004)
- ❖ Urinary catheter (p=0.04)
- ❖ Antibiotic exposure
  - ➢ mainly third-generation cephalosporins (p=0.00001)
- ❖ Transfer between hospital units (p=0.008)

✓ Zhao et.al in 2014: China

✓ Torres et al in 2015: Mexico



Discussion

- The predominant species found in our study was ***K. pneumoniae***, followed by ***E. coli***
- The $\boldsymbol{bla}_{\textbf{OXA-48}}$ was the most frequently detected carbapenemase and $\boldsymbol{bla}_{\textbf{NDM-1}}$ was the second rank
- Both carbapenemase producing *K. pneumoniae* and *E.coli* cluster I strains were isolated among inpatients who shared a room hence PFGE profile of the strains was identical. Therefore, it is possible that a spread of CPE from patient to patient occurred.

Discussion: Carriage

- Mainly hospitalized patients in the **ICUs** were screened
- Rate of carriage was high (37.9%)

✓ Higher than in a 2012 report from **Greece** (12.8) as well as one from **Korea** (0.3)

❖ Hand hygiene non-compliance, breaches in environmental sanitation in all hospital areas (especially in the ICU), increased duration of hospitalization and extensive use of broad-spectrum antimicrobial agents

- CRE multiple colonization in the same patient (15 patients)

✓ Intra- and inter-species transmission of bla_{NDM} (IncA/C, IncFII) and $bla_{\text{OXA-48}}$ (IncL/M) genes within the gut microbiome.

❖ Rapid identification of **CRE carriages** could be an important strategy to control the transmission of these organisms in healthcare facilities

Conclusion

- **Clonal dissemination** and **outbreak** of OXA-48 and NDM-1 producing *K. pneumoniae* has been observed.
  - **ST11, ST893, ST147**
- **Detection of high risk clones** (ST11 *K. pneumoniae* and ST131 E. coli)
  - high capacity to colonize and persist over time
- **Co-expression** of carbapenemase genes together reduces the options of treatment
- Alert on the large dissemination of these genes to other hospitals and community
- So, we must therefore focus on rapid identification of CPE colonized patients and implementation effective infection control measures.

Conclusion: Other CRE isolates

➢ ***Serratia marcescens***

✓ 5 isolates collected from Tehran

✓ Probably the routes of transmission from patient to patient are either by:

1. direct contact through carriage of CRE on the hands of HCWs
2. indirectly via contaminated environmental surfaces or shared equipm

➢ ***Proteus mirabilis***

✓ 4 isolates collected from Esfahan

➢ ***Enterobacter cloacae***

✓ 2 isolates collected from Tehran

❖ The potential dissemination of ***P. mirabilis*** and ***S. marcescens*** OXA-48 producer isolates is a major problem, because this organism is intrinsically resistant to colistin

Conclusion

- Our study, **provides novel information** about the presence and distribution of carbapenemases among the CRE isolates in Iran

- High rate of CRE intestinal colonization and CRE infection among inpatients:

1. Might be related to the close relationship between Iran and neighbor countries such as Pakistan, Turkey, Afghanistan and Iraq in terms of population exchanges

2. The lack of knowledge of hospital personnel about these bacteria

Prevention

- An aggressive infection control and prevention strategy is recommended, including reinforcement of **hand hygiene**, using **contact precautions** and **early detection** of ESBL-E and CPE through use of targeted surveillance


DESTRIPTION
Destination & Trip Description


Thank you for your attention