AMRDETECTOOL
Detection in 30 min

CLINICAL IMPACT OF THE USE OF RAPID DIAGNOSTIC TEST

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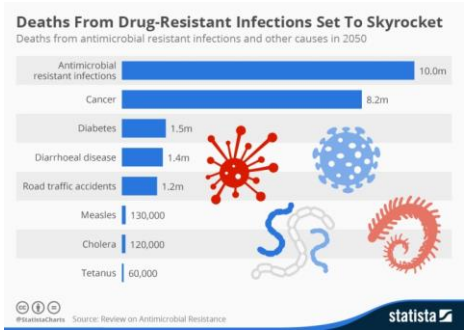
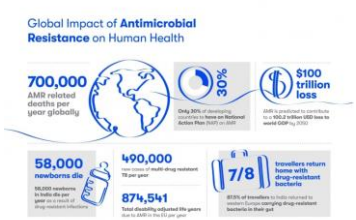
Antimicrobial resistance

- ✓ Public health problem
- ✓ Specially urgent in **bacteria**
- ✓ The World Health Organization (WHO): global effort
- ✓ Impact of antibiotic resistance: CDC data





Detection in 30 min

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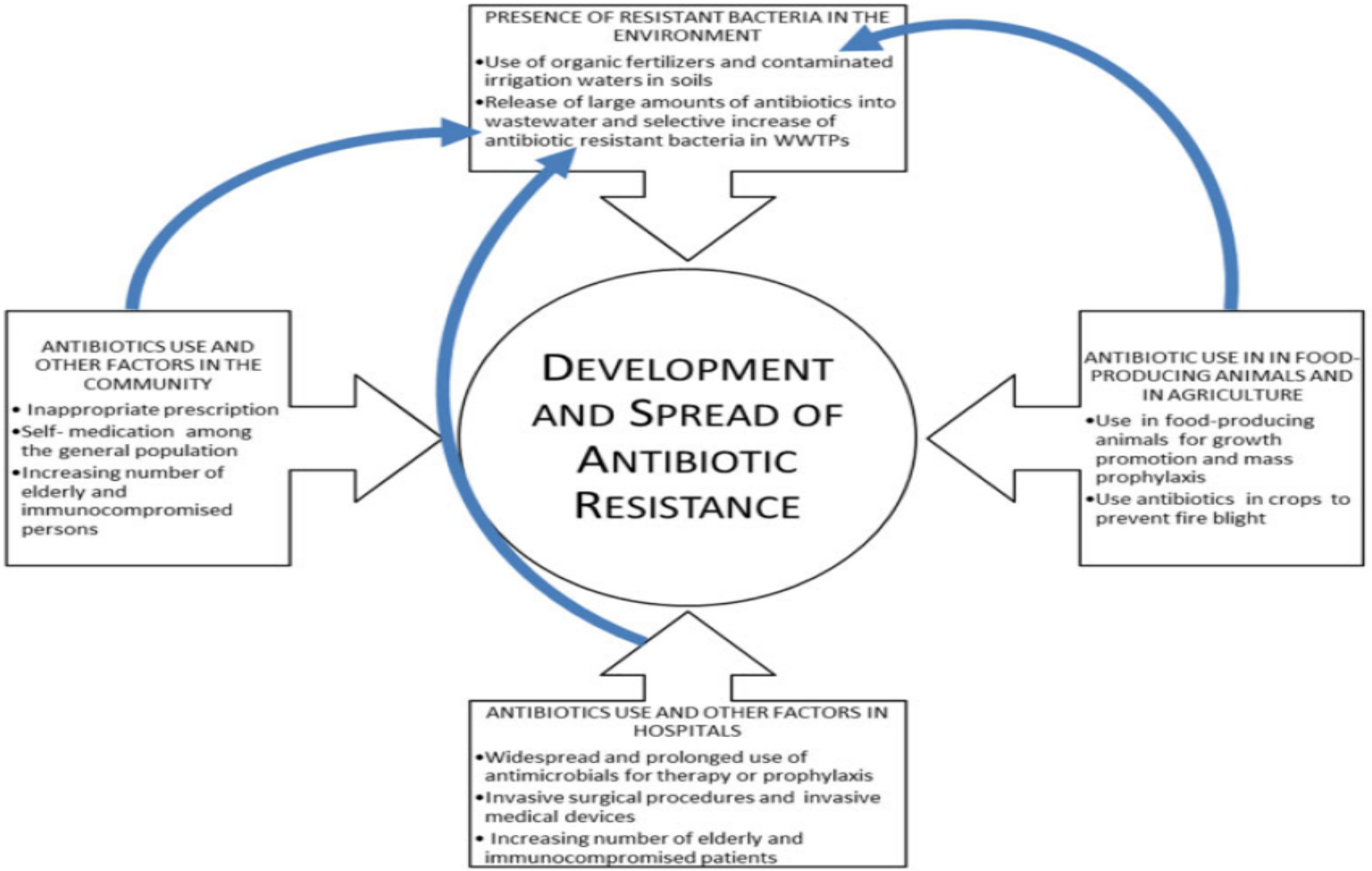

Antimicrobial resistance: a global multifaceted phenomenon Francesca Prestinaci, Patrizio Pezzotti, Annalisa Pantosti-DOI 10.1179/2047773215Y.0000000030.2016.

Detection in 30 min

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Strategies against antibiotic resistance

Developing stronger surveillance systems in both human and animal sectors

Strengthening healthcare systems and laboratory infrastructure: providing equipment, training staff and facilitating sample collection

Supporting a worldwide exchange of information

Enhancing data reporting, monitoring, and evaluation: generating evidence-based guidelines and solutions

Detection in 30 min

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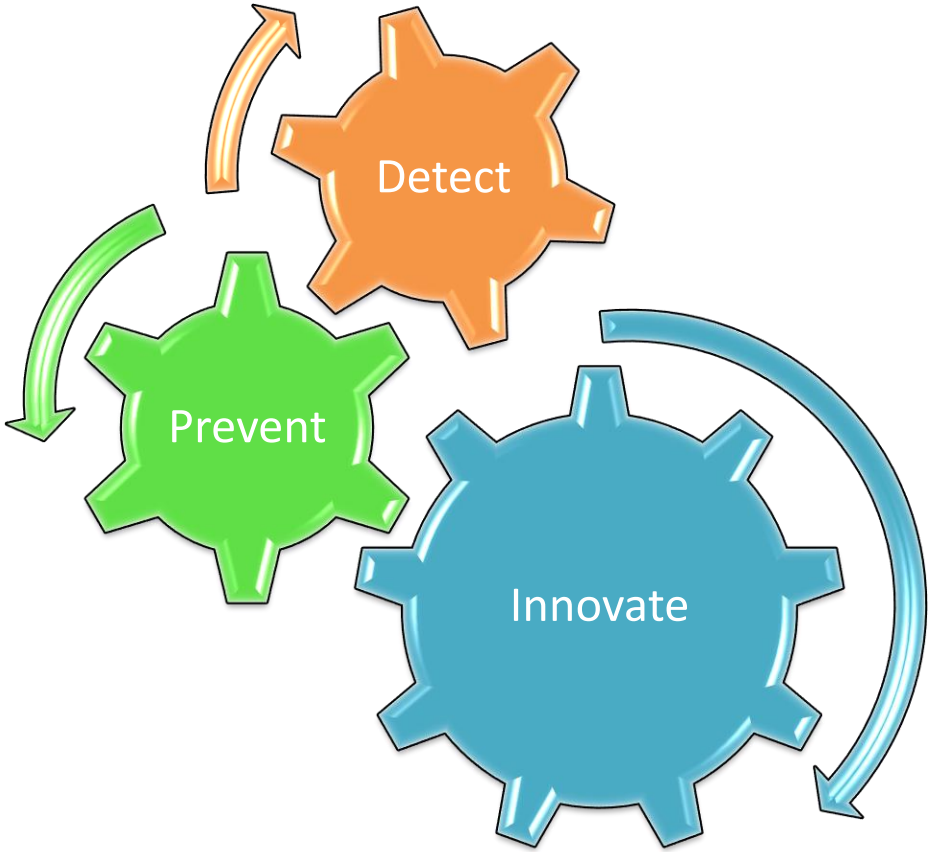

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Epidemiology

Urinary tract infection (UTI) is one of the most common bacterial infections.

The diagnosis of a UTI can be made by a combination of symptoms and a positive urine analysis or culture.

The differentiation between uncomplicated and complicated UTIs has implications for therapy because the risks of complications or treatment failure are increased for patients with a complicated UTI.

Uncomplicated UTI is an episode of cystitis in a woman who is not pregnant, is not immunocompromised, has no anatomical and functional abnormalities of the urogenital tract, and does not exhibit signs of tissue invasion and systemic infection.

Asymptomatic bacteriuria (ASB), which is defined as the presence of a positive urine culture with at least 105 cfu/ml collected from a patient without symptoms of a UTI, is a common, but usually benign phenomenon.

Foxman B. Nature Reviews 2010; Geerlings S. Microbiology Spectrum 2016;

Epidemiology

- In a 2004–2005 laboratory based surveillance of community acquired UTI in Canada: 40,618 UTI episodes were recorded → Annual incidence of 17.5 episodes per 1,000 people.
- Our hospital in numbers:
  - Tertiary care hospital – 700 hospital beds
  - 5 episode per day – 1825 episodes per year
  - 1 monographic outpatient clinic
    - 4 new visits everyday
    - 15 patients follow-up per day

Epidemiology

- SMART study
- 937 UTI episodes
- Multicenter study (10 hospitals in Spain)

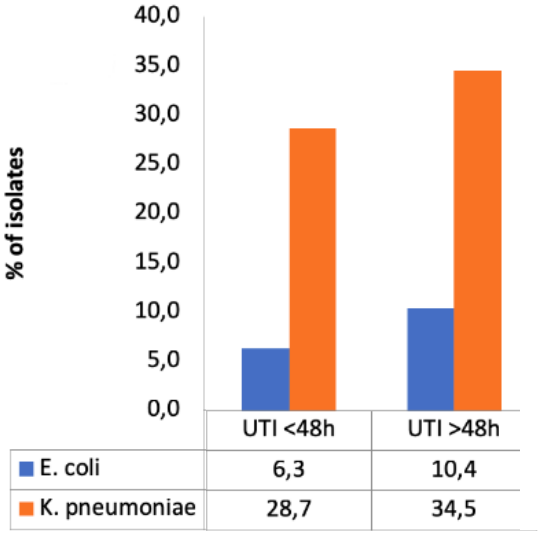
63,3% *E. coli*
16,7 % *Klebsiella spp.*
6,9% *Proteus mirabillis*

Table 2 Distribution of the most common Gram-negative organisms collected in urinary tract infections in Spain in the SMART Study (2016-2017).

		Community associataed		Nosocomial associated	
Organisms	No. isolates	No.	%	No.	%
Escherichia coli	504	284	63.3	220	44.9
Klebsiella pneumoniae	205	66	14.7	139	28.4
Klebsiella oxytoca	18	9	2.0	9	1.8
Proteus mirabilis	61	31	6.9	30	6.1
			1.1	11	2.2
			1.3	5	1.0
			1.7	13	2.6
			0.6	4	0.8
Other Enterobacterales	33	13	2.9	20	4.0
Pseudomonas aeruginosa	57	22	4.9	35	7.1
Other Gram-negative bacilli	4	1	0.2	3	0.6
TOTAL	937	448	47.8	489	52.2

Canton et al. Rev Esp Quimioter, 2019

Epidemiology



	UTI <48h	UTI >48h
E. coli	6,3	10,4
K. pneumoniae	28,7	34,5

Figure 1 Percentage of *Escherichia coli* and *Klebsiella pneumoniae* isolates with extended spectrum β-lactamases by origin of acquisition of infection in the SMART study in Spain comparing intra-abdominal (IAI) and urinary tract infections (UTI) infections.

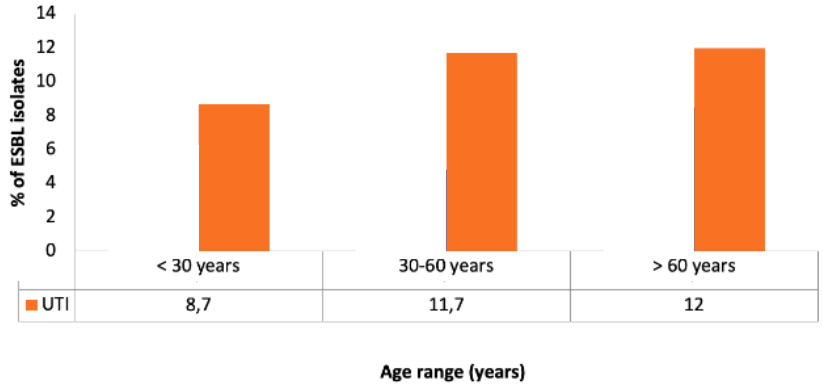
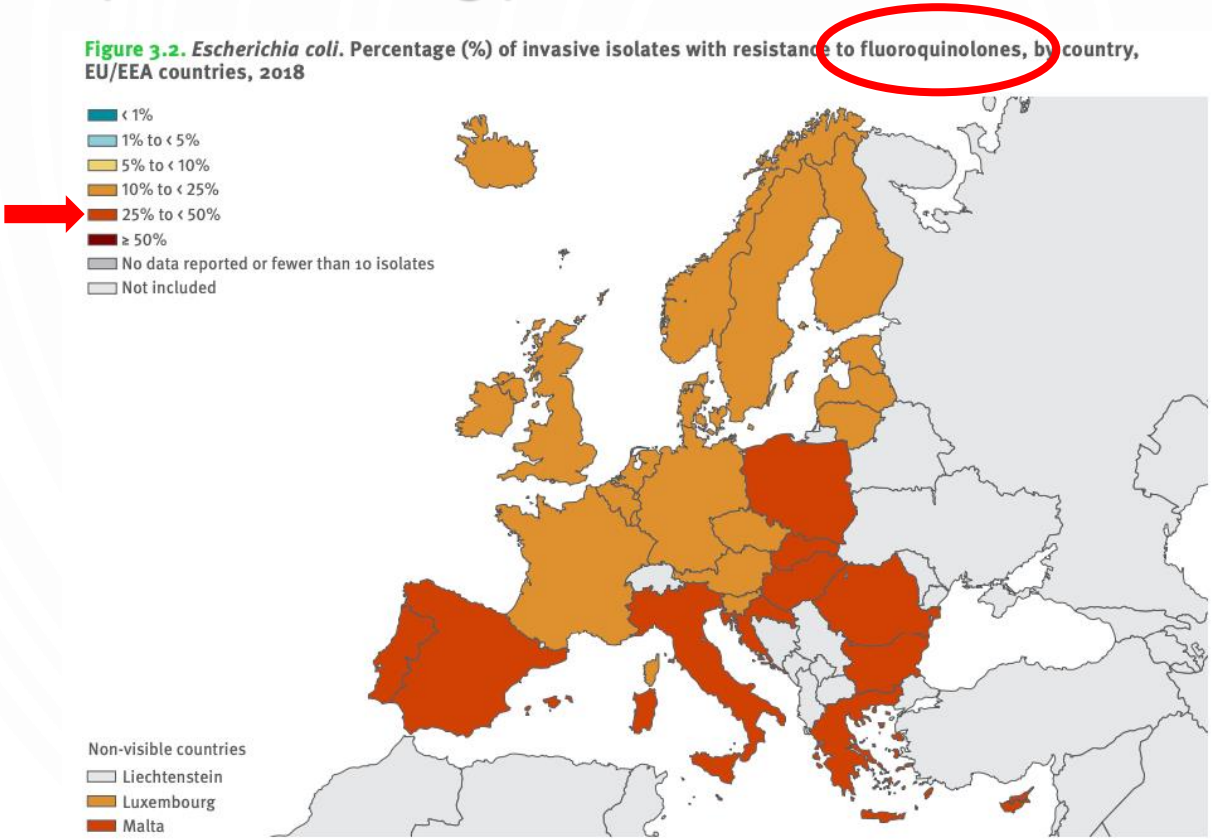


Figure 2 Frequency of Enterobacterales (*Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca* and *Proteus mirabilis*) with extended spectrum β-lactamases according to age of the patients in the SMART study in Spain comparing intra-abdominal (IAI) and urinary tract infections (UTI) infections.

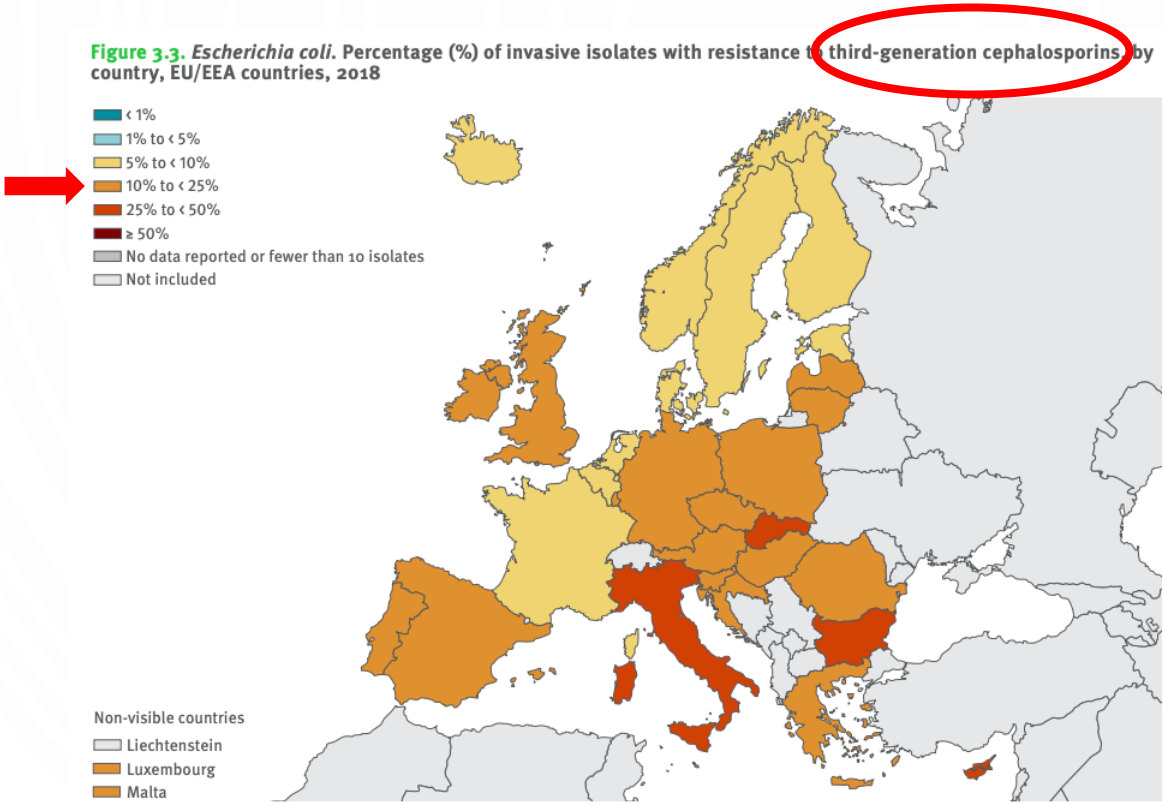
Modfied from Canton et al. Rev Esp Quimioter, 2019

Epidemiology



ECDC. Surveillance report, 2018

Epidemiology



ECDC. Surveillance report, 2018

Epidemiology and prognostic determinants of bacteremic acute pyelonephritis in women
Journal of Infection 2013

The aim of this study was to determine the epidemiology of bacteremic APN in women during 1991-2010 in our setting and to identify the independent prognostic factors of mortality in these cases.

An infectious disease specialist and one microbiologist review the charts of patients with positive blood cultures and recommend antibiotic therapy according to the clinical context and the results of the Gram's stain organism identification and antimicrobial susceptibility test.

Patients were observed from the diagnosis of bacteraemia until 30 days afterwards, until death in hospital or until discharge.

Epidemiology and prognostic determinants of bacteremic acute pyelonephritis in women
Journal of Infection 2013

Mean age	65 years
Comorbidities	Diabetes mellitus, Solid organ cancer
mmunity- adquired	89%
ck on presentation	9%
Mortality	4%

Epidemiology and prognostic determinants of bacteremic acute pyelonephritis in women
Journal of Infection 2013

Table 1 Microorganisms most frequently isolated in bacteremic APN.

Microorganisms	*N* (%)	Community-acquired	Healthcare-associated	1991–1995	1996–2000	2001–2005	2006–2010
Total	2238	1985	253	382	640	668	548
E. coli	1887 (84)	1713 (86)	174 (69)	307 (80)	554 (87)	573 (86)	453 (83)
FQ-R	337 (18)[a]	283 (14)	54 (21)	20 (7)[a]	70 (13)[a]	120 (21)[a]	127 (28)[a]
CTX-R	45 (2)[a]	35 (2)	10 (4)	–	4 (1)[a]	11 (2)[a]	30 (7)[a]
Klebsiella spp.	119 (5)	84 (4)	35 (14)	14 (4)	19 (3)	35 (5)	51 (9)
FQ-R	20 (17)[a]	8 (<1)	12 (5)	–	–	4 (11)[a]	16 (31)[a]
CTX-R	12 (10)[a]	0 (–)	12 (5)	–	–	2 (6)[a]	10 (20)[a]
P. mirabilis	107 (5)	98 (5)	9 (4)	31 (8)	28 (4)	32 (5)	16 (3)
FQ-R	14 (13)[a]	11 (1)	3 (1)	3 (10)[a]	1 (4)[a]	6 (21)[a]	4 (25)[a]
Enterococcus spp.	35 (1)	21 (1)	14 (6)	11 (3)	7 (2)	9 (1)	8 (2)
Enterobacter spp.	27 (1)	19 (1)	8 (3)	2 (1)	6 (1)	8 (1)	11 (2)
P. aeruginosa	18 (1)	11 (1)	7 (3)	6 (2)	3 (1)	2 (<1)	7 (1)

FQ-R: Fluoroquinolone resistant; CTX-R: Cefotaxime resistant. Other microorganisms that represent less than 5% of isolates are: *S. aureus*, *Citrobacter* spp., *Acinetobacter* spp., *Serratia* spp., other *Pseudomonas* spp.

[a] Percentage of specific microorganism.

Epidemiology and prognostic determinants of bacteremic acute pyelonephritis in women
Journal of Infection 2013

FACTORS ASSOCIATED WITH MORTALITY

- AGE
- ULTIMATELY OR RAPIDLY FATAL PROGNOSIS OF UNDERLYING DISEASE
- HEALTH-CARE ASSOCIATED INFECTION
- SHOCK ON PRESENTATION
- **AREK ISOLATE**
- **INAPPROPRIATE EMPIRICAL ANTIBIOTIC TREATMENT**

RISK FACTORS OF INFECTION WITH AN AREK STRAIN

Previous antibiotic therapy	Health-care associated bacteraemia

WHAT SHOULD I DO?

I GOT IT, I WILL GIVE TO ALL MY PATIENTS CARBAPENEM EMPIRIC TREATMENT

Efficacy and safety of carbapenems versus new antibiotics for treatment of adult patients with complicated urinary tract infections: A systematic review and meta-analysis

Open Forum Infectious Diseases, 2020

To evaluated the clinical efficacy and safety of carbapenems for the treatment of cUTIs with the comparators being new antibiotics evaluated for this indication.

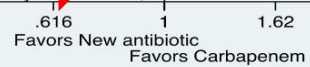
PICOS CRITERIA

- Participants: adult participants (age 18 years and over) with complicated urinary tract infections
- Intervention: carbapenem treatments
- Comparison: new antibiotic treatments (defined as those submitted to FDA or EMA for approval for the cUTI indication from 2009 to 2019, excluding carbapenems alone or in combination with new beta-lactamase inhibitors).
- Outcomes: efficacy and safety measures, including clinical response, microbiological response, and adverse effects.
- Study type: randomised controlled trials.

(A) Composite Cure

Study	Carbapenem events	Carbapenem total	New antibiotic events	New antibiotic total	RR (95% CI)	% Weight
Vazquez, 2012	21	35	18	27	0.90 (0.62, 1.32)	8.74
Wagenlehner, 2016	269	417	280	393	0.91 (0.82, 1.00)	24.43
Portsmouth, 2018	65	119	183	252	0.75 (0.63, 0.90)	18.61
Wagenlehner, 2019	138	197	156	191	0.86 (0.77, 0.96)	23.24
IGNITE3	302	403	293	[illegible]	1.09 (1.00, 1.19)	24.99
Overall (I-squared = 81.1%, p = 0.000)					0.91 (0.79, 1.04)	100.00

NOTE: Weights are from random effects analysis



(B) Clinical Response

Study	Carbapenem events	Carbapenem total	New antibiotc events	New antibiotc total	RR (95% CI)	% Weight
Vazquez, 2012	29	36	24	28	0.94 (0.75, 1.17)	1.82
Carmeli, 2016	129	137	132	144	1.03 (0.96, 1.10)	21.25
Wagenlehner, 2016	377	417	355	393	1.00 (0.96, 1.05)	43.73
Portsmouth, 2018	104	119	226	252	0.97 (0.90, 1.06)	13.81
Wagenlehner, 2019	178	197	170	[illegible]	1.02 (0.95, 1.09)	19.38
Overall (I-squared = 0.0%, p = 0.830)					1.00 (0.97, 1.03)	100.00

NOTE: Weights are from random effects analysis

(C) Microbiological Response

Study	Carbapenem events	Carbapenem total	New antibiotic events	New antibiotic total	RR (95% CI)	% Weight
Vazquez, 2012	31	49	31	46	0.94 (0.70, 1.26)	5.54
Carmeli, 2016	88	137	118	144	0.78 (0.68, 0.91)	17.46
Wagenlehner, 2016	291	417	299	393	0.92 (0.84, 1.00)	33.67
Portsmouth, 2018	67	119	184	252	0.77 (0.65, 0.92)	13.36
Wagenlehner, 2019	147	197	171	191	0.83 (0.76, 0.92)	29.96
Overall (I-squared = 33.7%, p = 0.197)					0.85 (0.79, 0.91)	100.00

NOTE: Weights are from random effects analysis

Efficacy and safety of carbapenems versus new antibiotics for treatment of adult patients with complicated urinary tract infections: A systematic review and meta-analysis

Open Forum Infectious Diseases, 2020

New antibiotics can perform with similar clinical efficacy and safety but may have a better microbiological response compared to carbapenems for the treatment of patients with cUTIs.

In settings where there is a risk of a carbapenem-resistant pathogens, these new antibiotics could be an important therapeutic option.

AMRDETECTOOL
Detection in 30 min

NG-TEST®CTX- M Multi: multiplex lateral flow immunoassay for the rapid detection of prevalent ESBL CTX-M Groups (1, 2, 8, 9, and 25).

NG-TEST® Carba 5: multiplex lateral flow immunoassay for the rapid detection and differentiation of KPC, OXA-48, VIM, IMP and NDM carbapenemases.

31st ECCMID Online 9 – 12 July 2021

ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

Implementation of the direct identification and resistance detection from urine in routine diagnostic procedure

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CLÍNIC BARCELONA Hospital Universitari

INTRODUCTION

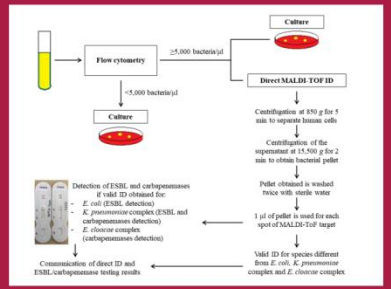
Urinary tract infection is a common disease in primary care and hospital settings. The current gold standard for the diagnosis of urinary tract infection (UTI), including culture and antimicrobial susceptibility testing, requires at least 36 h. Rapid diagnosis of UTI can improve patient management and prognosis, especially for patients with bacteriaemia and sepsis from urinary source.

AIM

The aim of this study was to evaluate the implementation of a combination of flow cytometry for screening, mass spectrometry (MALDI-TOF) for direct identification (ID) followed by rapid detection of ESBL and carbapenemases with bacterial pellet obtained from urine.

METHOD

We analysed 298 samples processed between June and December 2020. Only samples from patients attended in Emergency Department with suspicion of bacteriaemia of urinary origin were included. Flow cytometry was used for screening. Urines with bacterial count of ≥5,000 bacteria/μL were processed for direct MALDI-TOF ID. Direct ESBL and carbapenemase detection was performed, using NG-Test CTX-M MULTI and NG-Test CARBA 5 (NG biotech, France), if following species were reliably identified: *Escherichia coli*, *Klebsiella pneumoniae* complex and *Enterobacter cloacae* complex. Clinicians were informed about results immediately and treatment was adjusted if necessary.



RESULTS

Results of analysed samples are shown in the Figure 1. Of the total of 298 samples processed, there were 141 (47.3%) culture negative, 114 (38.3%) culture positive, and 43 (14.4%) contaminated. Positive Predictive Value of direct ID for 86 samples that achieved bacterial count of ≥5,000 bacteria/μL was 96.4%. From 114 samples positive according to culture results, 54 (47.4%) were correctly identified the same day of sample receipt. Figure 2 shows results of 54 samples with concordant reliable direct ID. One discordant result was obtained: direct ID positive for *Lactobacillus crispatus* whereas in urine culture *K. pneumoniae* and *Candida albicans* were isolated. There was a reliable direct ID for *E. coli* from one urine sample considered as contaminated by culture. Of the 212 samples that did not meet the criterion of ≥5,000 bacteria/μL, only 36 were culture positive , representing 31.6% of the total of positive samples. It should be noted that 22/36 (61.1%) samples showed bacterial count of $<10^5$ CFU/mL by culture.

Figure 1. Results of 298 samples analysed

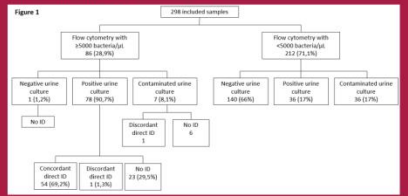


Figure 2. Results of 54 samples with reliable direct ID

Figure 2

Direct ID by MALDI-TOF (number of samples)	Mean direct MALDI-TOF score (min – max)	Urine culture results (number of samples)
E. coli (30)	2214 (1861 – 2498)	*E. coli* (27), *E. coli* + *Enterococcus faecalis* (1), *E. coli* + *K. pneumoniae* complex (1) and *E. coli* + *Pseudomonas aeruginosa* (1)
K. pneumoniae complex (10)	2189 (1898 – 2430)	*K. pneumoniae* complex (8), *K. pneumoniae* complex + *P. aeruginosa* (1) and *K. pneumoniae* complex + *Enterococcus faecium* (1)
P. aeruginosa (3)	2018 (1770 – 2145)	*P. aeruginosa* (2) and *P. aeruginosa* + *K. pneumoniae* complex (1)
Klebsiella aerogenes (2)	2367 and 2394	*K. aerogenes* (2)
Klebsiella oxytoca (2)	2291 and 2377	*K. oxytoca* (2)
Proteus mirabilis (2)	1857 and 2232	*P. mirabilis* (2)
Morganella morganii (1)	2305	*M. morganii* (1)
E. cloacae complex (1)	2221	*E. cloacae* complex (1)
E. faecalis (1)	2334	*E. faecalis* (1)
E. faecium (1)	2089	*E. faecium* + *E. faecalis* (1)
Aerococcus urinae (1)	1883	*A. urinae* (1)

Table 1. Results of 27 cases with bacteriaemia from urine origin

Blood culture (n)	Urine culture (n)	MALDI-TOF direct ID (n)	Direct detection of ESBL and carbapenemases (n)	Conventional susceptibility testing results (n)
E. coli (19)	*E. coli* (19)	*E. coli* (11)	ESBL detected (4/11)	ESBL detected (4/11)
		- No ID (6) - Bacterial count ≤5,000 bacteria/μL (2)	NA	ESBL detected (3/8)
K. pneumoniae complex (4)	*K. pneumoniae* complex (2) *K. pneumoniae* complex + *E. coli* (1) *K. pneumoniae* + *C. albicans* (1)	*K. pneumoniae* complex (2)	ESBL detected (1/2)	ESBL detected (1/2)
		- No ID (1) - Discordant ID (*Lactobacillus crispatus*)(1)	NA	ESBL not detected (2/2)
P. aeruginosa (1)	*P. aeruginosa* (1)	Bacterial count ≤5,000 bacteria/μL	NA	NA
E. cloacae complex (1)	*E. cloacae* complex (1)	No ID (1)	NA	NA
P. mirabilis (1)	*P. mirabilis* (1)	*P. mirabilis* (1)	NA	NA
S. aureus (1)	*S. aureus* (1)	Bacterial count ≤5,000 bacteria/μL	NA	NA

Ten ESBL-producing strains were detected by lateral flow performed with bacterial pellet, including 6 *E. coli* and 4 *K. pneumoniae* complex. There was not any carbapenemase-producing bacterium during analysed period. The concordance between ESBL and carbapenemase detection and routine susceptibility testing was 100%. The treatment was adjusted according to results of direct testing: in four out of 10 cases with ESBL positive test empirical ceftriaxone was changed to ertapenem.

The same microorganism was recovered from blood and urine in 27 cases from de total of 238 patients analysed (Table 1). Remarkably, 14/27 (51,8%) were cases of bacteriaemia with reliable direct ID obtained from urine the same day of sample receipt.

CONCLUSIONS

The proposed protocol presents the following strengths for diagnosis of UTI:

- Allows rapid identification of urine pathogens (the same day of sample receipt);
- Allows to identify microorganisms that grow with difficulty in culture media and/or standard conditions of incubation;
- Allows to apply a rapid direct detection of ESBL and/or carbapenemase-producing strains according to local epidemiology, once a reliable ID is obtained;
- Allows early diagnosis of more than 50% of cases with bacteriaemia from urine origin;
- The protocol could be combined with other methods of screening (automated urine sediment analyzers or Gram staining) to detect high bacterial inoculum, and other rapid techniques available in laboratories for bacterial resistance detection.

REFERENCES

Zboromyrska Y *et al*. Development of a new protocol for rapid bacterial identification and susceptibility testing directly from urine samples. *Clin Microbiol Infect. 2016; 22:561.e1-6.*

Zboromyrska Y *et al*. A multicentre study investigating parameters which influence direct bacterial identification from urine. *PLoS One. 2018; 13:e0207822.*

ACKNOWLEDGEMENTS

We are grateful to all colleagues who made possible the implementation of this protocol during the COVID-19 pandemic

CONTACT INFORMATION

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LAB ROUTINE IN URINE SAMPLES

Urine test strip - Culture

Identification and Antimicrobial Susceptibility Testing (AST)

Detection in 30 min

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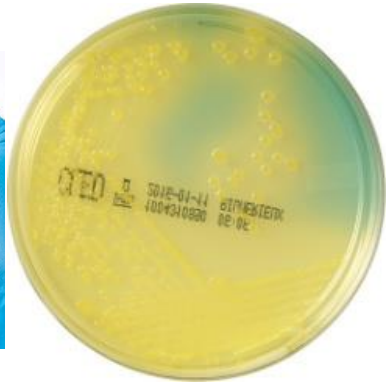
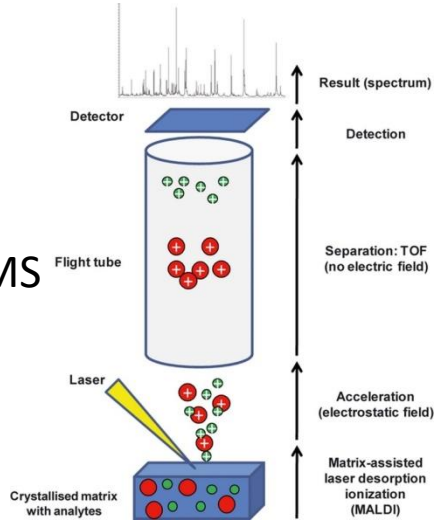
NEW WORKFLOW



UF-4000 (Sysmex, Kobe, Japan)
Flow cytometry

Direct ID

MALDI TOF- MS



Detection in 30 min

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Antimicrobial Susceptibility Testing

- AST Method:

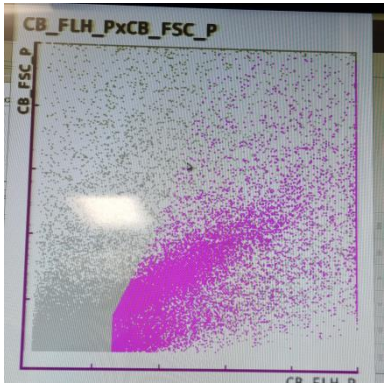
✓ Disc diffusion

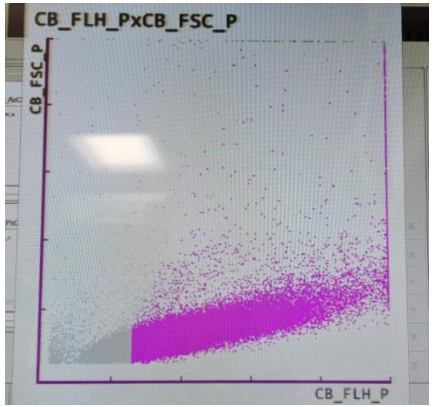
✓ Interpretation according to the European committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines

Flow cytometry selection criteria

✓≥5.000 bacteria/µL

✓ Scattergram bacterial differentiation





Detection in 30 min

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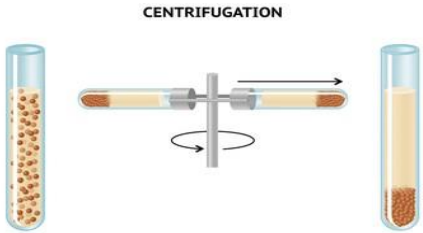
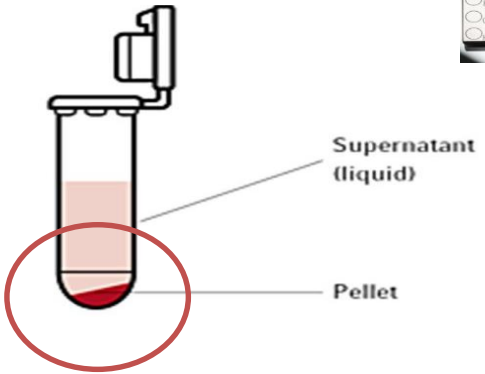
Workflow evaluation

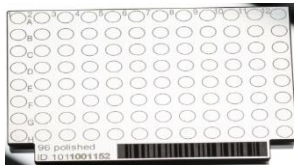
Direct identification

✓ MALDI-TOF-MS-based identification

10 mL







Score ≥2.0 indicates specie identification

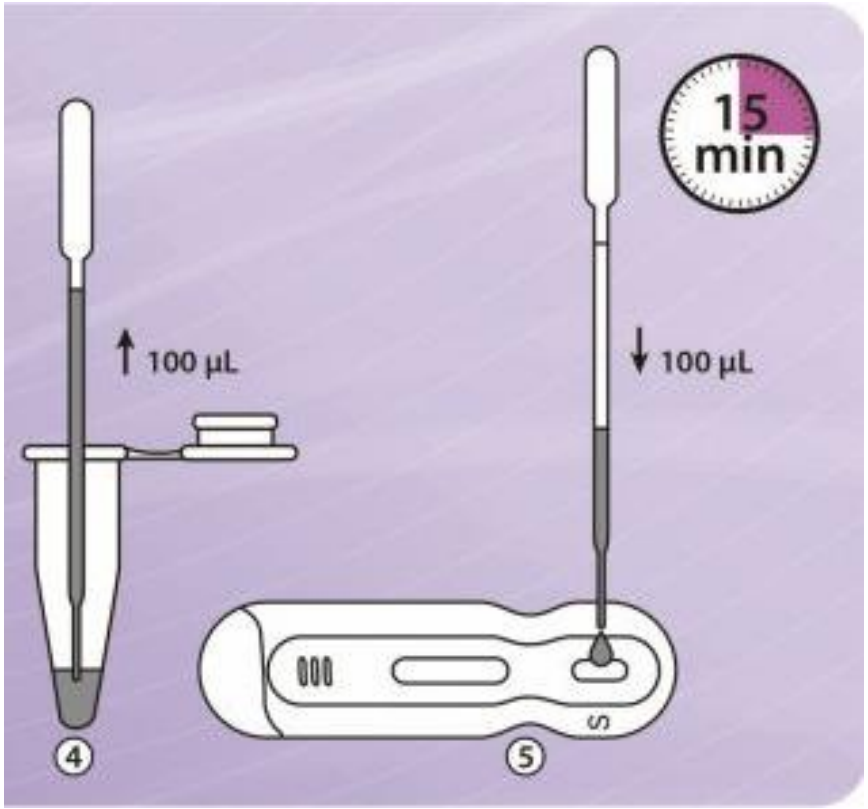
Detection in 30 min

AMRDETECTOOL

Workflow evaluation

<u>Resistance detection (ESBL-carbapenemase)</u>

- ✓ MALDI-TOF MS reliable identification:
- *E. coli*
- *K. pneumoniae complex* (*K. pneumoniae, K. quasipneumoniae and K. variicola)*
- *E. cloacae complex (E. asburiae, E. cloacae, E. hormaechei, E. kobei y E. ludwigii)*
- ✓ NG-TEST: CTX-M and Carba 5



Detection in 30 min

AMRDETECTOOL

Workflow evaluation

Results report

- The flow cytometry and MALDI-TOF MS results were validated in our lab informatic system
- Clinician in charge: reviewed patient's medical history and treatment

Detection in 30 min

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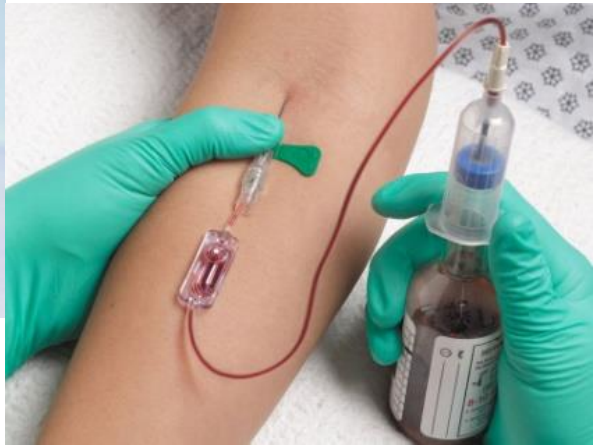
Workflow evaluation

Samples

- ✓ 298 urine samples were analysed (June-December 2020)
- ✓ Emergency room
- ✓ Blood culture



Detection in 30 min

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Workflow evaluation

Sample inclusion criteria

- ✓Sample reception: 00:00 – 15:00 time frame
- ✓Urine volume ≥ 10 mL
- ✓ Flow cytometry criteria

Detection in 30 min

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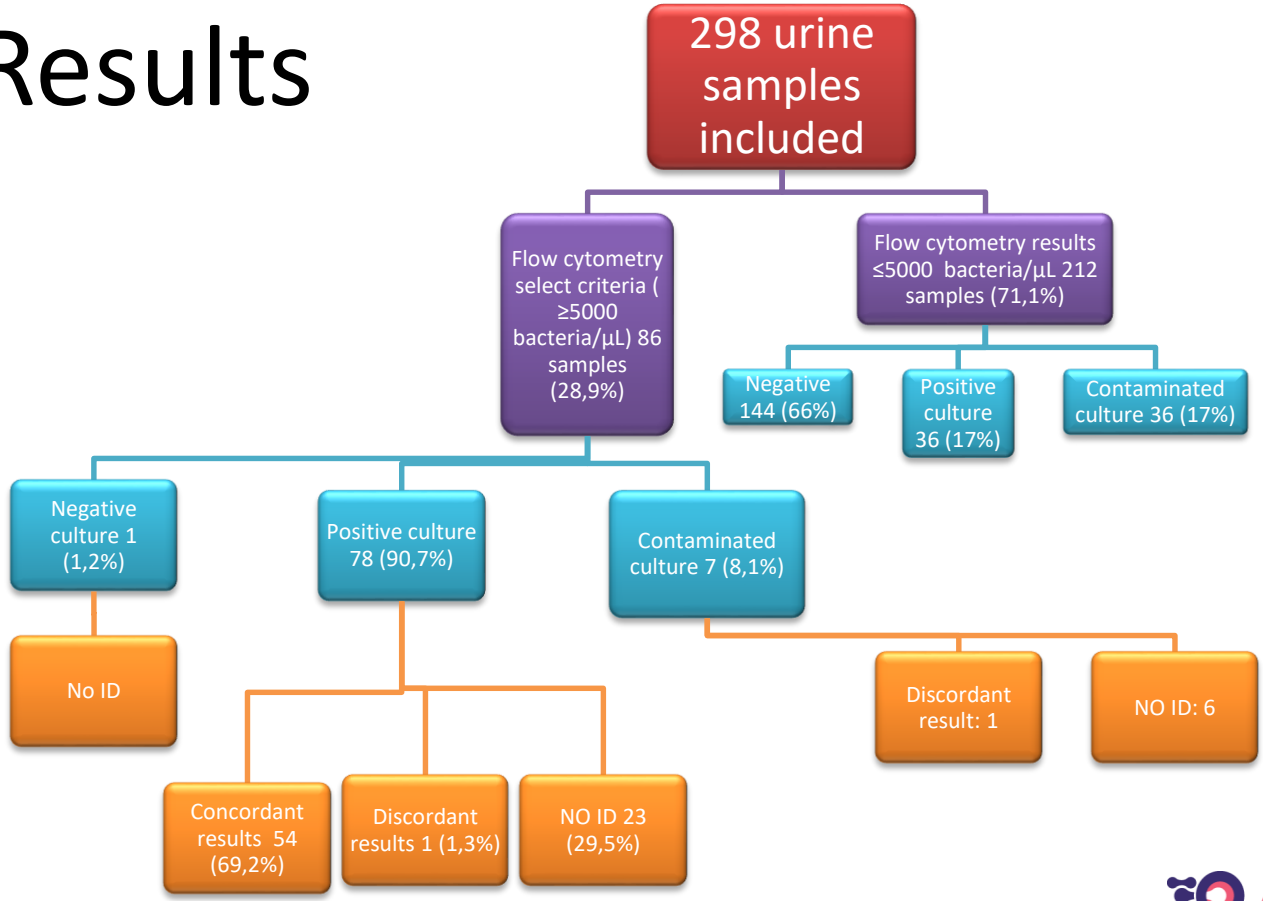
Results



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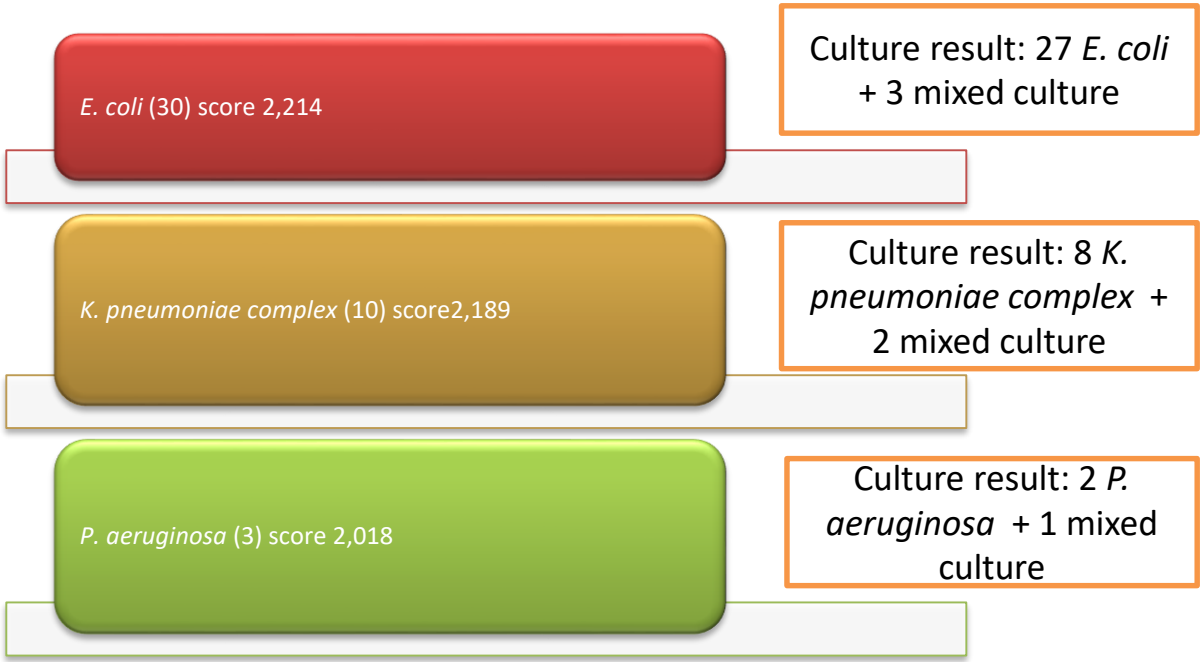
Results



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Results

Direct ID	MALDI-TOF MS score (minimum and maximum value)	Culture results
K. aerogenes (2)	2,367-2,394	*K. aerogenes* (2)
K. oxytoca (2)	2,291-2,377	*K. oxytoca* (2)
P. mirabilis (2)	1,857-2,232	*P. mirabilis* (2)
M. morganii (1)	2,305	*M. morganii* (1)
E. cloacae complex (1)	2,221	*E. cloacae complex* (1)
E. faecalis (1)	2,334	*E. faecalis* (1)
E. faecium (1)	2,089	*E. faecium* + *E. faecalis* (1)
A. urinae (1)	1,883	*A. urinae* (1)

Detection in 30 min

AMRDETECTOOL

Results

- ✓ No reliable ID: 23 cases
- ✓ Discordant case: direct identification *E. coli* – mixed culture considered as contamination
- ✓ Flow cytometry result: ≤ 5.000 bacteria/µl 212 cases (36 positive culture)
- ✓ A total of 22/36 positive cultures presented a bacterial count < 10^5 UFC/mL

Detection in 30 min

AMRDETECTOOL

Results

Direct ID: 54 cases

36 cases NG test were performed

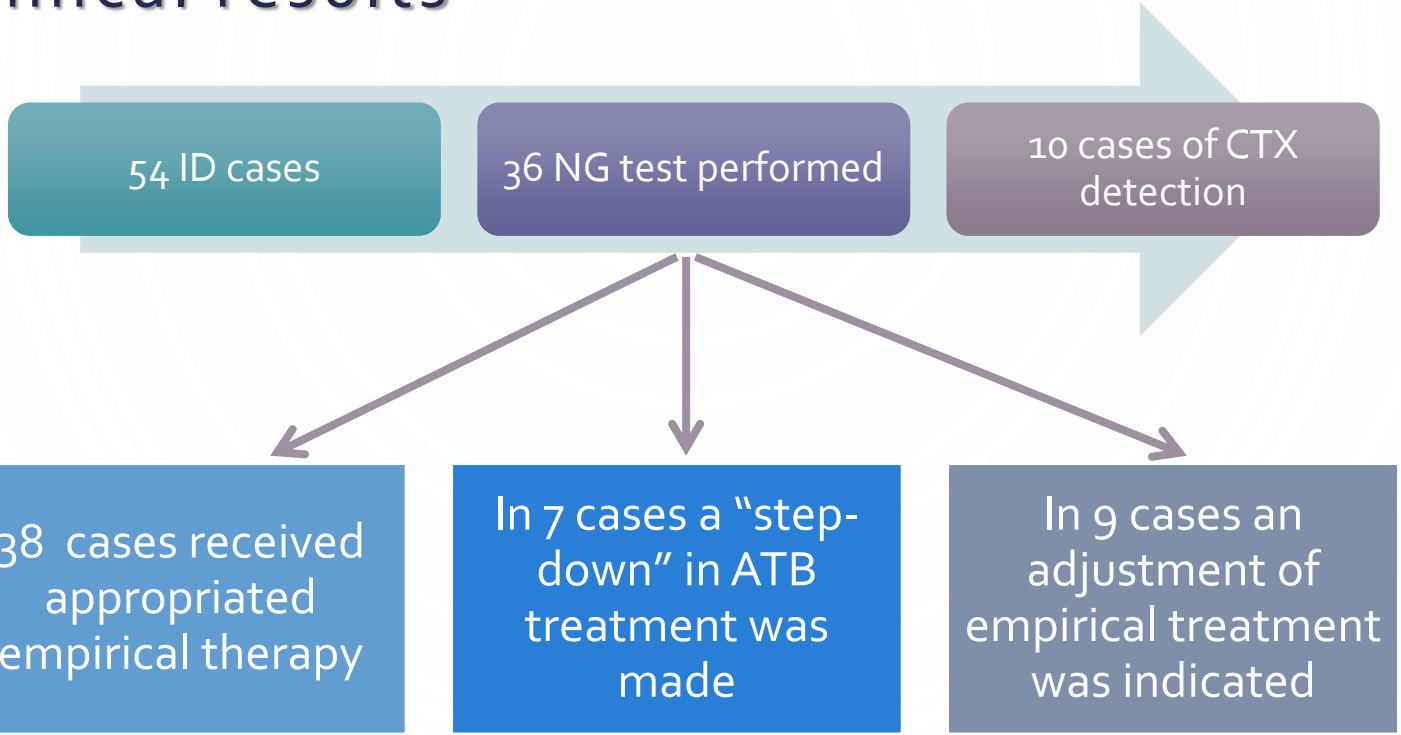
100% agreement with conventional AST

CTX-M detection: positive in 10 cases. *E. coli* (6) and *K. pneumoniae complex* (4). No carbapenemases were detected

Detection in 30 min

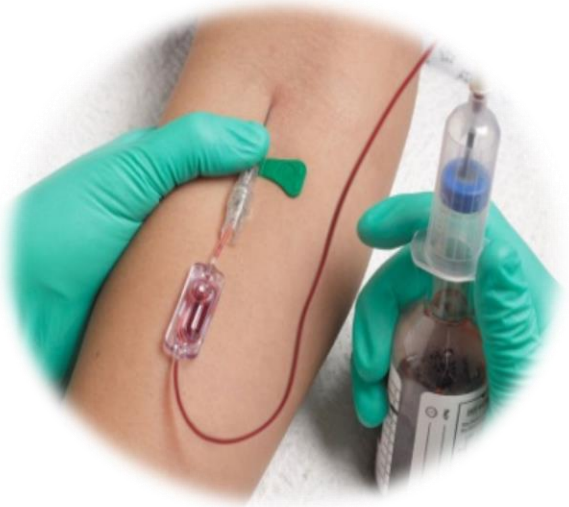
AMRDETECTOOL

Clinical results



OVERALL, THE USE OF CARBAPENEMS WAS REDUCE IN 7,4%

Results



- 298 blood cultures
- 27 positive samples
- 14/27 direct identification
- 8/27 no reliable identification
- 4/27 <5.000 µL/mL (1 discordant case)

Detection in 30 min

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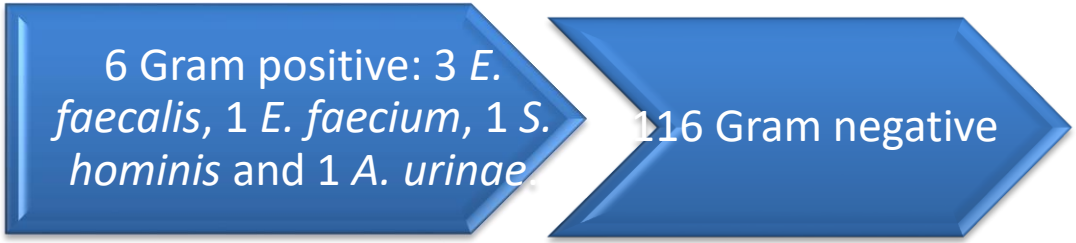
Workflow implementation results

- ✓ Six months of experience (January-June 2021)
- ✓ 122 samples included (120 patients)
- ✓ Emergency room patients (80) and hospitalized (42)



Detection in 30 min

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Workflow implementation results

116 Gram negative

67 E.coli (15 BLEE: 22,4%)
32 K.pneumoniae (12 BLEE: 37,5% / 3 BLEE+CARBA (2 OXA 1 VIM): 9,4%
6 P.aeruginosa
4 P.mirabilis
2 E.cloacae (2 negative CARBA)
2 P.putida (2 CARBA: VIM)
1 C.freundii (Negative ESBL)
1 C.koseri (Negative ESBL)
1 K.oxytoca (Negative ESBL)

- Correct ID: 96 cases
- 5 contaminated cultures
- 14 mixed cultures

Detection in 30 min

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NG test results

ESBL+ CARBA: 122 test
Not performed in 16 cases
Negative tests: 74
ESBL detected: 27 cases
CARBA detected: 2 cases (2 VIM)
ESBL + CARBA detected: 3 (2 OXA 1 VIM)

Detection in 30 min

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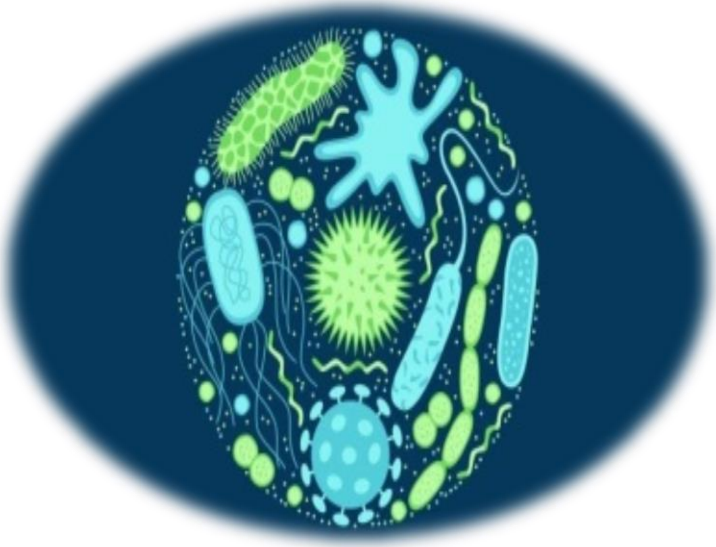
Summary



- ✓ Flow cytometry + MALDI TOF MS for direct identification reach a hight agreement with urine culture
- ✓ The lateral flow assay for ESBL and carbapenemases has an important clinical impact

Detection in 30 min

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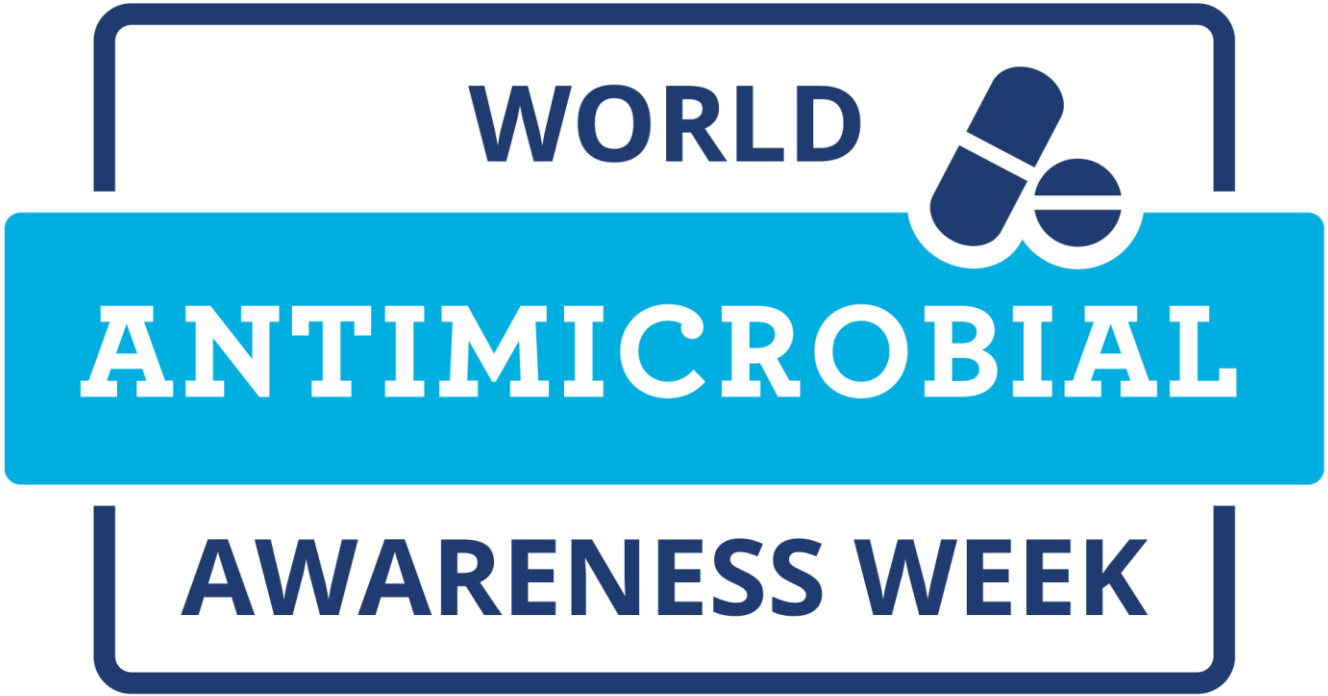

Detection in 30 min

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Detection in 30 min

THANK YOU
FOR YOUR ATTENTION!

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eit Health
EIT Health is supported by the EIT, a body of the European Union