

Resistance of uropathogens to antibacterial agents: Emerging threats, trends and treatments

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Summary

Urinary tract infections are among the most common infectious diseases in humans.

Today, resistance to nearly all antimicrobial classes is dramatically growing, and extremely drug-resistant or even pan-drug resistant pathogens are increasingly isolated around the world. It is foreseen that in the next decades the world will be facing a major medical emergency generated by the rapid spread of pathogens carrying resistance determinants of unprecedented power. Carbapenemase-producing Enterobacteriaceae, multidrug-resistant Enterococci and fluoroquinolone resistance determinants in both Gram-negative and Gram-positive uropathogens are among the greatest emergencies. In this article, the major emerging threats of particular interest to urologists are reviewed, worldwide resistance trends are illustrated, and novel and older – but still active – recommended drugs are summarized.

KEY WORDS: Urinary tract infections; Resistance; Antibacterial agents; Antibiotics; Carbapenemase; Carbapenem resistance.

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INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial-borne diseases in humans. Although antibacterial agents have been the mainstay of treatment for UTIs for decades, today routine antibiotic therapy is seriously threatened by worldwide outbursts of infections involving multidrug-resistant (MDR), extremely drug-resistant (XDR) or even pan drug-resistant pathogens. Gram-negative Enterobacteriaceae like *Klebsiella pneumoniae* (KP) and *Escherichia coli* (EC), and Gram-positive cocci like *Enterococcus faecalis* and *Enterococcus faecium* are at the same time the most common uropathogens and the bacteria carrying the most powerful resistance determinants. On the basis of a general assessment of the major resistance threats, the present review will focus on worldwide susceptibility trends resulting from large international surveillance studies, and will present some available therapeutic options – either novel or old but still effective – for the management of resistant infections of urological interest.

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EMERGING RESISTANCE THREATS

In 2013, the Centers for Disease Control and Prevention (USA) included carbapenem-resistant Enterobacteriaceae (CREB) among the three microorganisms posing an urgent threat to public health, due to the extensive resistance shown by these pathogens to a wide array of antibacterial agents, and to the very high mortality rates reported in patients with bloodstream infections caused by organisms like carbapenem-resistant KP. Several international programs are now aimed at fostering research, development and awareness about the threat of pathogen resistance. Pivotal European trials are for example EURECA (European prospective cohort study on Enterobacteriaceae Showing Resistance to Carbapenems), and REVISIT (Revisiting serious bacterial infection with innovation), promoted in the frame of the European COMBACTE-CARE project, an initiative of the combacte.com collaboration.

Carbapenem resistance

For several decades, extended-spectrum beta-lactamases (ESBL) of TEM and SHV lineage, conferring resistance to a variety of penicillins and third-generation cephalosporins, have represented a worrisome problem for physicians treating patients affected by infectious diseases. In these cases, carbapenem therapy was a useful therapeutic resource against ESBL-producing pathogens. However, today the usefulness of carbapenems is decreasing rapidly, and the global spread of carbapenemase-producing Enterobacteriaceae (the etiological agents of lung, soft tissue and urinary tract infections) has undoubtedly become the most important threat in the field of infectious diseases. The speed of such spread is alarming: for example, whereas in 2009 the spread of the *Klebsiella pneumoniae carbapenemase* (KPC) in the USA was classified as 'sporadic' in half of the federal states and 'less than sporadic' in the remaining states (1), in 2014 the entire USA territory has been defined as affected by an 'endemic' presence of KPC (2). The concern raised by this mounting trend is due to the

fact that the spectrum of enzymatic activity of carbapenemases is not restricted to drugs like imipenem, ertapenem, meropenem or doripenem, but extends to almost all beta-lactams. Moreover, resistance to carbapenems and other beta-lactams is in most cases facilitated by horizontal-transfer of genetic elements like transposons and plasmid-borne integrons, invariably containing multiple resistance determinants which often confer multidrug-resistant, extensively drug-resistant or even pan drug-resistant properties to pathogens.

K. pneumoniae is the most prevalent pathogen among carbapenem-resistant *Enterobacteriaceae* according to a recent surveillance report by the *European Centre for Disease Prevention and Control* (3). Infection with carbapenemase-producing *KP* (CPKP) can double the mortality rate of affected patients (from 21% to 42%, all infections), and can triplicate the mortality of non-intensive care patients with UTIs from 13% to 43% in *intensive care unit* (ICU) cases (4).

The carbapenemases that have been characterized so far belong to all four Ambler classes of beta-lactamases (Table 1). Among the enzymes of concern for urologists, the class-A, plasmid-borne *K. pneumoniae* serine carbapenemase (KPC) is today the most common transmissible resistance determinant in *Enterobacteriaceae*. KPC includes more than 20 variants (the most prevalent being KPC-2 and -3), who can hydrolyze virtually all beta-lactam agents including carbapenems, penicillins, broad spectrum cephalosporins and monobactams. KPCs are only weakly inhibited by clavulanate and tazobactam (1, 5), and the encoding blaKPC gene, predominantly present in IncF plasmids (with FIIK replicons, and often associated with a Tn4401 trasposon-like structure), is very often co-expressed with cotrimoxazole, fluoroquinolone and aminoglycoside resistance determinants (6).

Notably, today KPC is no longer exclusively expressed in *KP* (mainly the ST258 strain) or other CREB, but is also found in other species such as *Pseudomonas aeruginosa* (7). The SME enzyme also belongs to

class-A carbapenemases, but at present is isolated less frequently than KPC, being in almost all cases restricted

Table 1.
Carbapenem resistance determinants in pathogens involved in UTIs.

Ambler Class carbapenemases	Resistance determinant	Expression	Activity	Susceptibility to inhibitors	Co-expressed resistance determinants
A	<i>K. pneumoniae</i> serine carbapenemase (KPC, >20 variants)	<i>K. pneumoniae</i> (mainly the ST258 strain) other <i>Enterobacteriaceae</i> <i>P. aeruginosa</i>	carbapenems penicillins broad spectrum cephalosporins monobactams	Avibactam, relebactam, vaborbactam; weak to clavulanate and tazobactam	trimethoprim-SMX fluoroquinolones aminoglycosides
B	SME enzyme Metallo beta-lactamases (MBLs, e.g. New Delhi MBL (NDM), Verona-Integron-Encoded MBL (VIM), IMP imipenemase	<i>Serratia</i> spp. <i>Eiterobacteriaceae</i> <i>P. aeruginosa</i>	carbapenems penicillins cephalosporins, (not monobactams)	Not affected	other carbapenemases & beta-lactamases aminoglycosides fluoroquinolones macrolide esterases trimethoprim-SMX
C	CMY enzymes (e.g., ACT-1, CMY-1, CMY-2, CMY-4)	<i>Eiterobacter</i> spp. other <i>Eiterobacteriaceae</i> (including <i>K. pneumoniae</i>)	moderate carbapenemase activity, increased by impaired drug permeability (e.g., OmpK porins)	Avibactam, vaborbactam; not significantly affected by clavulanate	/
D	OXA beta-lactamases (e.g., OXA-23, OXA-48, OXA-51 OXA-58)	<i>K. pneumoniae</i> , <i>E. coli</i>	weak carbapenemase activity, increased by concomitant activity of efflux pumps or low-permeability porins	Avibactam, relebactam	/
Mechanisms in Gram-positive pathogens	Decreased affinity of carbapenems to penicillin-binding proteins (e.g., PBP4), acquired by single or multiple point-mutation	<i>Eiterococcus</i> spp.	/	/	/

to the *Serratia marcescens* species, a member of the *Enterobacteriaceae* family (8).

Class B metallo beta-lactamases/carbapenemases (MBLs) require zinc for their catalytic activity. These enzymes are expressed in *Enterobacteriaceae* but also in *P. aeruginosa*, and can hydrolyze carbapenems, penicillins and cephalosporins, but not monobactams. Importantly, MBLs are not affected by beta-lactamase inhibitors.

The most powerful and most diffuse MBLs are the *New Delhi MBL* (NDM), the *Verona-Integron-Encoded MBL* (VIM) and the IMP imipenemase (9). The blaNDM gene variants are harbored by a variety of broad-host-range Inc plasmids like IncA/C, IncF, IncR, IncN, IncM, IncX, which are in turn expressed within various pathogen isolates, including the highly prevalent ST11 KP clone. Pathogens harboring the NDM are often residually susceptible to a single last-resort agent like polymyxin E, since NDM is very frequently co-expressed with other carbapenemases (OXA-48, VIM and/or KPC) and with multiple resistance determinants such as ESBL, AmpC beta-lactamases, aminoglycoside-modifying enzymes, fluoroquinolone resistance enzymes (e.g., Qnr), macrolide esterases, as well as determinants conferring resistance to trimethoprim-sulphamethoxazole.

Among class C beta-lactamases, the CMY enzymes (e.g., ACT-1, CMY-1, CMY-2, and CMY-4, often transferred via pYMG plasmids) were originally expressed in *Enterobacter* spp. but now are being carried by several other *Enterobacteriaceae*, including KP (CMY-10). ACT-1 and CMY-1 show moderate carbapenemase activity, are not significantly inhibited by clavulanic acid, and high-level resistance to carbapenems is often the result of combined drug hydrolysis and impaired drug permeability (e.g., involving OmpK35/36 porins) (10, 11).

Class D OXA beta-lactamases can efficiently hydrolyze penicillins like oxacillin. Some OXA enzymes, such as OXA-23, OXA-48, OXA-51 and OXA-58, carried mainly by highly transferable IncL group plasmids like pOXA-48a, have a weak carbapenemase activity. Nevertheless, in pathogens like ST-11 KP, high-MIC carbapenem resistance may result from the concomitant activity of efflux pumps or low-permeability porins (12).

As far as carbapenem-resistance in Gram-positive pathogens is concerned, three mechanisms of reduced beta-lactam susceptibility have been reported in Enterococci: (i) beta-lactamase production as well as (ii) overproduction or (iii) inactivation (by point-mutation) of penicillin-binding proteins like PBP4 or PBP5. Notably, carbapenem resistance is mainly caused by point-mutations in PBP4. For example, resistance to faropenem in *E. faecalis* is due to decreased affinity of the drug for PBP4, due to the acquisition of one or two point mutations in the PBP4-encoding gene (13).

Plasmid-mediated fluoroquinolone resistance

Plasmid mediated fluoroquinolone resistance (PMQR) is increasing to the point that international guidelines no longer recommend drugs like ciprofloxacin, ofloxacin or levofloxacin as first-choice agents for treatment of urinary tract infections, when resistance is assessed in at least 10% of pathogen isolates (14, 15).

Fluoroquinolones (FQ) inhibit the activity of bacterial

gyrase and topoisomerase IV in Gram-negative and -positive pathogens. Mutations occurring in the quinolone resistance determining regions of genes encoding these type II topoisomerases (e.g., in gyrA, gyrB, parC or parE subunits) are the most common chromosomal determinants of FQ resistance (16). For example, in Enterococci resistance to fluoroquinolones is mainly caused by mutations in the GyrA and ParC genes in gyrase and topoisomerase IV, respectively (17). Other mechanisms of resistance to FQ are the qepA-encoded efflux pumps, the OqxAB-encoded efflux pumps -very common in plasmids carried by *K. pneumoniae*-, the qnr-encoded proteins, impeding the interaction of FQ with DNA gyrase, and a mutant aminoglycoside acetyl-transferase (aac (6')-Ib-cr) which acetylates the piperazine ring of FQ like norfloxacin and ciprofloxacin (18-21).

Determinants of PMQR are horizontally transferable. For example, qnrA1, A3, A6, B2, B4, B6 and B10 are associated with the mobilizing element insertion sequence ISCR, whereas qnrB1 and B20 are associated with IS26 and Orf1005. The aac (6')-Ib-cr enzyme-encoding gene is often found in association with qnrB and blaCTX in a cassette within an IS26 transposon. In addition, qepA and OqxAB are also often mobilized by IS26 transposons (22).

Polymyxin resistance

Polymyxins (polymyxin B and colistin/polymyxin E) are antibiotics produced by the Gram-positive species *Paenibacillus polymyxa*. The plasmidic mcr-1 colistin resistance gene, expressed in *Klebsiella* spp. and other *Enterobacteriaceae*, encodes for a phosphatidylethanolamine transferase enzyme, lessening the affinity of colistin towards the lipid-A on bacterial cell membranes via enzymatic modification (23). This resistance determinant, rapidly spreading worldwide (24), represents a major threat since polymyxins are considered last-resource antibiotics against CREB.

Glycopeptide resistance

Together with *Enterobacteriaceae*, Enterococci are the most common etiological determinants of urinary tract infections. Acquired resistance to glycopeptide antibiotics in Enterococci is given by five gene clusters: vanA, vanB, vanD, vanE and vanG, leading to the expression of peptidoglycal pentapeptide precursors (PPP) characterized by poor affinity for vancomycin and other glycopeptides. Interestingly, vanA Enterococci are resistant to the last-generation glycopeptide dalbavancin but are susceptible to oritavancin, possibly due to the unique dual mechanism of action of the latter (25). Horizontal transfer of Van genes may occur via Tn-1546 transposons, which are found in conjugative and non-conjugative plasmids (26).

WORLDWIDE RESISTANCE TRENDS

Beta-lactam antibiotic resistance

Information about worldwide trends in pathogen resistance is not frequently published, due to the fact that global epidemiological studies are difficult to perform, and most studies focus on a single nation or region. Nevertheless, quality works like for example the 2016

systematic review by Lee *et al.* (27), or some global surveillance studies, which are occasionally performed, give access to evidence concerning global trends of pathogen chemoresistance.

The *Study to Monitor Antimicrobial Resistance Trends* (SMART) is among the largest global surveillance programs aimed at monitoring longitudinal antimicrobial resistance patterns worldwide. Over 200,000 clinical samples have been collected since 2002 from patients with complicated intra-abdominal infections, whereas isolates from patients with UTIs have been acquired since 2009. Among other surveillance actions, susceptibility testing to 12 commonly used antibacterial agents has been performed in different regions of the world. One-hundred ninety-four hospital sites in countries located within the macro-regions of *Asia/Pacific*, *Latin America*, *Middle East/Africa*, *North America*, and *Europe* have taken part in the project. Within the SMART program, the ESKAPE group of pathogens (*Enterococcus spp.*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp.*) is of main interest to urologists and accounts for the vast majority of resistance encountered in nosocomial settings.

Global trends. Data from the SMART project provide an excellent overview of global resistance trends for uropathogens. In the frame of SMART, the 2009-2010 susceptibility analysis of *E. coli* isolates from UTI specimens of hospitalized patients in countries worldwide showed an overall 17.9% prevalence of *extended-spectrum beta lactamase* (ESBL) resistance determinants (28), whose rate varied depending on the region considered (*Asia/Pacific*, 27.7%, *Latin America*, 23.3%, *Europe*, 18.8%, *Middle East/Africa*, 16.2%, *Northern America*, 7.4%).

Only imipenem and ertapenem demonstrated > 90% susceptibility in ESBL-positive *E. coli* at that time (99.7% and 98% isolates susceptible, respectively), whereas susceptibility to amikacin and piperacillin-tazobactam was lower (87.1% and 84.4%, respectively). The least active agents were ampicillin-sulbactam, all cephalosporins except cefoxitin, and the fluoroquinolones (28). In the discussion of their data, Hoban *et al.* emphasized the relevance of intermediate susceptibility in the treatment of UTIs, as certain antibacterial agents concentrate physiologically in the urine (28). This suggests that in specific infections showing intermediate susceptibility towards certain agents, high-dose treatment may be attempted in the absence of more suitable options.

As far as the geographic distribution of carbapenemases is concerned, the overall mortality due to CREB reported in studies from *Northern America*, *Southern America*, *Europe*, and *Asia* was 33.2%, 46.7%, 50%, and 44.8%, respectively (all infections) (4).

KP carbapenemases (KPC) are endemic in the *USA*, *China*, *Greece*, *Italy*, *Poland*, *Israel*, *Brazil*, *Argentina*, *Colombia* and *Taiwan*, whereas sporadic spread of KPC-producing KP has been observed in virtually all Asian, European and American countries (summarized in: 27).

The *New Delhi metallo beta-lactamases* (NDM) confer resistance to most beta-lactams including carbapenems, as well as co-resistance to aminoglycosides, tetracyclines, fluoroquinolones and other antibacterials, due to the comp-

resence of various resistance determinants within the same mobile genetic elements. NDM are endemic *India*, *Pakistan* and *Bangladesh* (27). An analysis of the prevalence of NDM in the in the frame of the SMART program for years 2008-2012 – published in 2015 – showed that isolates of nine different species of NDM-expressing *Enterobacteriaceae* were disseminated in *India*, *Serbia*, the *Philippines*, *Saudi Arabia*, *Guatemala*, *Vietnam* and *Georgia*, and that different variants of the NDM gene were compresent with CTX-M cephalosporinases (29). Moreover, *Enterobacteriaceae* producing the VIM and IMP carbapenemases (alone or co-expressed) have been found in *Greece*, *Italy*, *Spain*, the *Philippines*, *Turkey*, *Australia*, *Mexico*, *USA* and *India*, with most isolates concomitantly nonsusceptible to one or more agents like ampicillin-sulbactam, ceftriaxone, cefepime, ceftazidime, piperacillin-tazobactam, ciprofloxacin, amikacin, tigecycline and colistin (30). Importantly, KP isolates produced IMP-26 together with various VIM variants (VIM-1, 5, 26 or 27), whereas *E. coli* expressed IMP-1 alone (30). Class-D OXA carbapenemases are endemic in *India*, *Turkey*, *Morocco*, *Libya*, *Egypt* and *Tunisia*, and sporadic spreads have been documented throughout *Europe* and the *Middle-East* (summarized in: 27). OXA enzymes have been recently isolated in all continents.

Asia-Pacific. In the Asia-Pacific region, 60 centers from 31 countries provided susceptibility data, which were collected in years 2010-2013 and published in 2016 (31). *Uropathogenic E. coli* (UPEC) and KP were the most prevalent isolates. *China* showed the highest rates of ESBL, both in UPEC and KP (66.4% in 2010 and 59.9% in 2013 for UPEC; 60% in 2010 and 54.5% in 2013 for KP). Besides *China*, dramatic increases of ESBL-expressing UPEC were observed for example in *Thailand* (54.2% in 2013 vs. 36.8% in 2010) and *Hong Kong* (41.7% in 2013 vs. 25% in 2010), while ESBL-KP were substantially increased in *New Zealand* (45.5% in 2013 vs. 23.1% in 2010), *South Korea* (55.6% in 2013 vs. 37.5% in 2010) and the *Philippines* (61.3% in 2013 vs. 37.9% in 2010). In the same region, Jean *et al.* reported a significant increase of the annual rates of carbapenemase prevalence between year 2008 (0.07%) and year 2014 (1.1%), with the blaNDM-1 allele becoming more prevalent than the IMP-26 gene (32).

Latin America. In 11 Latin American countries, analysis of resistance trends in years 2013-2015, published in 2017, showed that ESBL-positive KP accounted for 46.6% of UTI isolates, substantially higher than the values assessed in SMART 2003 (14%). ESBL-positive rates of KP were higher (52.9%) in isolates from intensive care unit (ICU) compared to non-ICU patients (44.4%) (33). Ertapenem and imipenem showed slightly lower rates of resistance, compared to other beta-lactams (37.4% and 24.1%, respectively, versus, e.g., > 38% for cephalosporins and piperacillin-tazobactam). Among other antimicrobials tested, the aminoglycoside amikacin continues to show high rates of susceptibility (92.3%) for isolates of KP (33). **Northern America.** The SMART program provided 24655 isolates from Europe and North America, obtained from ICU (17.8%) and NON-ICU (82.2%) wards. Data from

90 hospitals in *Canada*, in the USA and in 18 European countries were collected. By comparing these regions, in 2015 Lob and coworkers found that resistance among *Enterobacteriaceae* in Europe was largely driven by KP expressing high rates of ESBLs (41.2% in intensive care units; mostly CTX-M) and carbapenemases (13.2%; mostly KPC and OXA-types). For all *Enterobacteriaceae* combined, only ertapenem and amikacin inhibited > 90% of ICU isolates in both regions. In Northern America, ertapenem, imipenem and amikacin inhibited > 90% of KP, whereas in Europe only amikacin attained such levels, according to Lob *et al.* (34).

In 2016, the same research group also focused on susceptibility patterns of ESBL in *E. coli* from UTIs, representing the 52% of Gram-negative pathogens collected. A significant increase in ESBL prevalence was seen in the US (from 7.8% in 2010 to 18.3% in 2014, $P < 0.0001$), whereas in *Canada* an increase from 10.4% to 13.0% was not statistically significant (35). Moreover, isolates from hospital-acquired UTIs increased considerably in the US (9.4% in 2010 to 27.7% in 2014). The steepest increases over the five-year study period were found among US males (from 7.1% to 26.2%) and older US patients (from 8.8% to 26.6%) (34).

Europe. National surveillance programs worldwide have reported extensive spreads of carbapenemase-producing uropathogens. Logan and Weinstein have edited maps showing the spread of CREB throughout Europe and the world (6). According to the 2017 survey published by the European Centre for Disease Prevention and Control (ECDC), 25% to 50% isolates of KP are carbapenem-resistant in *Italy*, *Romania* and *Greece*, the latter showing rates beyond the upper limit (36). In *Europe*, the diffusion of KPC is 'endemic' in *Greece*, *Poland* and *Italy* (1). From a study involving 3324 non-replicate isolates of *Enterobacteriaceae* from Italian hospitals, it was found that 4.3% of isolates were non-susceptible to carbapenems, *K. pneumoniae* being the most prevalent carrier of the blaKPC carbapenemase gene (in 25.1% and 7.7% inpatients and outpatients, respectively) (37).

A recent ECDC report focusing on *Italy*, pointed to dramatically increasing resistance trends in this country. For example, the proportion of CPKP blood isolates increased from 1.3% in 2006 to 33.5% in 2015, whereas combined resistance to third-generation cephalosporins, fluoroquinolones and aminoglycosides increased from 2.8% in 2005 to 29.7% in 2015. However, in *E. coli* the proportion of carbapenem-resistant blood isolates remained low: 0.1% in 2007 to 0.2% in 2015, though combined resistance increased from 0.8% in 2002 to 14.6% in 2015 (38). In *Germany*, a very low incidence of carbapenemase-producing isolates has been documented, as a multicenter study published in 2016 demonstrated an incidence of 0.047 cases per 1000 hospital admissions (39). Low prevalence has also been reported in *Belgium*, with 3.5% of *Enterobacteriaceae* being carbapenem non-susceptible through expression of OXA-48, OXA-427, KPC and NDM (40). A steep growth of carbapenem resistance has been reported in *France*. The OXA-48 and NDM resistance determinants increased from 23.1% in 2012 to 36.2% in 2014 (41).

Non-beta-lactam antibiotic resistance

As far as non-beta-lactam antibiotic susceptibility trends are concerned, certain African countries show dramatic prevalences of pan-resistant infections. For example, it has been reported that in *Nigeria uropathogenic E. coli* (UPEC) is pan-resistant to cotrimoxazole (100% isolates), and highly resistant to ofloxacin (70%), gentamycin (92%) and tetracycline (88%) (42).

In various *Asia-Pacific* countries, the SMART program reported that the rates of susceptibility to levofloxacin among hospital isolates of *E. coli* ranged from 83% in *New Zealand*, to 39% in *Singapore*, to 15% in *India* (cited in: 43). The overall prevalence of fluoroquinolone resistance in UPEC is dramatically increasing worldwide, with increasing trends in *Brazil* (63.53% increase for ciprofloxacin and 66.50% for norfloxacin from 2010 to 2015)(44), and with values up to 17% in *Italy* and 38% in *Turkey* (summarized in: 45), whereas in the US a 10-year study reported an increase from 3% to 17% between 2000 and 2010 (46).

In *Europe*, the areas showing the highest rates of PMQR are the Mediterranean countries. This distribution extends to the whole Mediterranean basin, and the affected countries are *Croatia*, *Slovenia*, *Greece*, *Italy*, *Spain*, *France*, *Morocco*, *Algeria*, *Tunisia*, *Egypt*, and *Turkey* (47, 48). Resistance to cotrimoxazole in community-acquired UPEC is high in *Southern America* (64% in *Nicaragua* and up to 58% in pediatric cases in *Brazil*), *Turkey* (up to 43%) and *Greece* (27.3%) whereas in the USA a percentage of 24.2 has been reported (45).

The prevalence of vancomycin-resistant *Enterococci* (VRE) causing symptomatic or asymptomatic infections in *Europe* ranges between 1 and 30%, whereas in the USA, VRE are about the 30% of all nosocomial isolates (49). Encouragingly, Toner *et al.* reported that the proportion of VRE has remained stable between 2006 (13.9%) and 2014 (11.5%), whereas a worrisome increase of resistance to nitrofurantoin has been documented in frame of the same study (just above zero in 2005 to over 40% in 2009 to about 20% in 2014). Notably, in that study the prevalence of vancomycin resistance was substantially higher in *E. faecium* (51.2%) compared to *E. faecalis* (1.6%)(49).

Despite the data mentioned above, uropathogens worldwide seem to retain sensitivity against agents like nitrofurantoin and fosfomycin. For example, the prevalence of resistance to nitrofurantoin in UPEC isolates has been reported to be 2.9% in *Brazil* (year 2007, 50), < 2% in *Europe* and 1.6 in the US (45, 49, 50). The figures concerning fosfomycin appear to be as low, with a prevalence in several European countries inferior to 2% (51, 52).

As far as infections of urological concern in the pediatric population are concerned, resistance to ciprofloxacin in *E. coli* UTIs increased 10-fold between 2002 and 2009 both in young boys and girls, as shown in a study performed in 195 US pediatric hospitals (1% to 10% and 0.6% to 4% of isolates, respectively) (53).

AVAILABLE AND EMERGING THERAPEUTIC STRATEGIES

Few randomized controlled studies concerning novel or improved therapeutic protocols against drug-resistant

infections have been performed so far. Thus, the available evidence is mainly observational and sometimes limited to case reports. In most cases, studies focusing on *Enterobacteriaceae* are based on complicated cases of pneumonia or bacteremia. Despite these limitations, this section will focus on selected therapeutic options -novel or old but still efficacious- for treatment of infections caused by resistant pathogens of concern to urologists.

Carbapenems

It is known that certain carbapenemase enzymes can decrease the susceptibility of pathogens to carbapenems to a limited extent, and PK/PD data suggest that $T > MIC$ targets can be met with high probability of attainment with high-dose continuous infusions of carbapenems (e.g. 6 g/day meropenem) when MICs are ranging between 4 and 16 mg/L (summarized in: 54).

These experimental data are encouraging, though experts suggest that monotherapy with these agents is not advisable, and that combination therapy including a carbapenem (when MICs are ≤ 8 mg/L) can result in lower mortality rates (54). Importantly, the efficacy of combined therapies including a carbapenem appears to be MIC-dependent, as mortality rates in sepsis patients infected with KPC-producing KP were up to 35% for MICs > 16 mg/L, but as low as 13.3% if the MICs of meropenem (2g, > 3 h infusion thrice daily) were below or equal to 4 mg/L (55).

Interestingly, combination of two carbapenems might become a last-resort regimen for treating pandrug-resistant and colistin-resort KPC-producing KP infections (bacteremia, pneumonia, and UTIs). The rationale for such approach is based on the fact that KPC appears to have higher affinity for ertapenem compared to other carbapenems. Thus, while KPC would be "engaged" by ertapenem, a co-administered different carbapenem could exert its bactericidal activity. Although recent quality observational data are encouraging (56-58), additional, adequately powered studies are urgently warranted. Moreover, such evidence may ideally foster the development of ertapenem analogues characterized by higher affinity for KPC compared to the founder compound, in order to optimally exploit such "engagement" activity on carbapenemases.

Aztreonam

Monobactam therapy may be considered as an option against *Enterobacteriaceae* expressing class B or D carbapenemases when these determinants are not co-expressed with class A enzymes, which are able to efficiently hydrolyze aztreonam (59). In all cases, susceptibility testing is necessary, due to variable monobactamase activity shown by different subclasses of class-B enzymes.

Avibactam

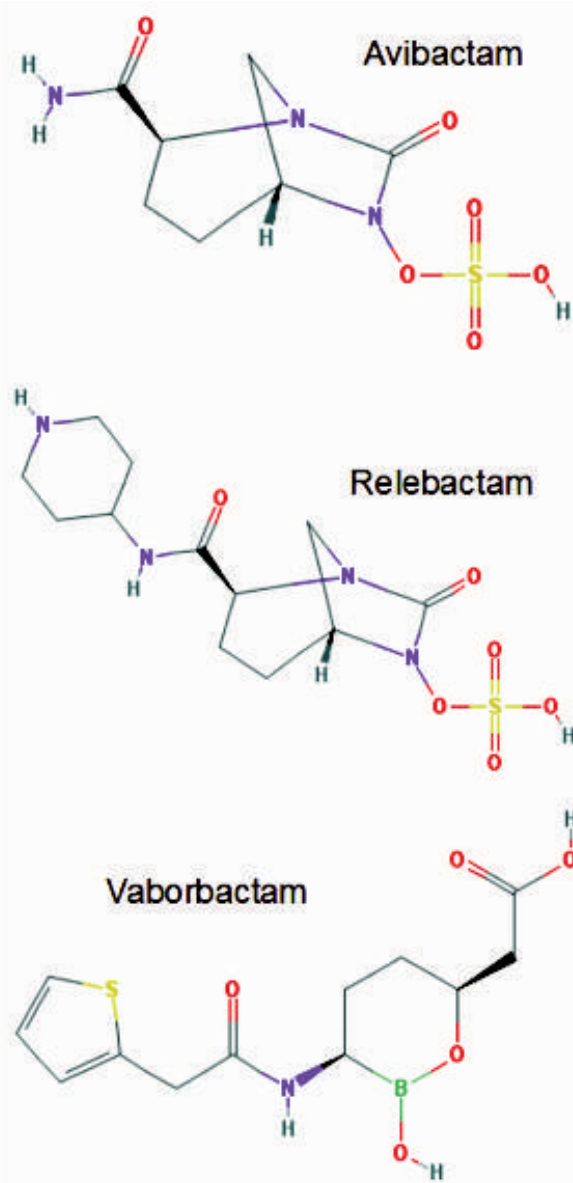
The novel non-beta-lactam beta-lactamase inhibitor avibactam (Figure 1) has been approved in 2016 in the European Union for treatment of soft tissue infections, pneumonia and urinary tract infections in combination with the third-generation cephalosporin ceftazidime. Avibactam inhibits a broad spectrum of beta-lactamases including A-class, C-class and some D-class carbapene-

mases (60). In vitro assessments performed in the frame of the INFORM global surveillance study demonstrated that 98% of CREB isolates containing KPC or OXA-48 enzymes were susceptible to this combination, even when the isolates expressed ESBLs or AmpC enzymes (61, 62). However, ceftazidime/avibactam was ineffective against carbapenem-hydrolyzing metallo-beta-lactamases like NDM.

In vitro activity of this combination was also demonstrated in the frame of a phase 3 trial involving ceftazidime-resistant UTIs (63). Recently, Jayol *et al.* investigated the in vitro activity of ceftazidime/avibactam, alone (for class A and D carbapenemase producers) or in combination with aztreonam (for class-B carbapenemase

Figure 1.

Chemical structure of the novel non-beta-lactam beta-lactamase inhibitors avibactam, relebactam and vaborbactam. Structures were drawn using the PubChem NIH public repository database (93).



producers), against a collection of colistin-resistant and carbapenemase-producing KP isolates (64).

It was shown that ceftazidime/avibactam was effective against colistin-resistant and KPC-producing or OXA-48-producing KP, and was also efficient against KP isolates co-expressing two carbapenemases. Interestingly, the combination of ceftazidime/avibactam with aztreonam was synergic against NDM-producing KP.

Investigators suggested that such synergic activity could be explained by the neutralization of the ESBL activity by avibactam, which can in turn restore the susceptibility of KP to aztreonam (64). These data warrant urgent clinical investigation.

The clinical efficacy of the ceftazidime/avibactam combination (2g/0.5g, intravenous q8h) was demonstrated by recent phase 3 studies in patients with complicated UTIs, including acute pyelonephritis (65, 66). Further studies are urgently needed to investigate the clinical cure rates of this combination in UTIs caused by CREB.

Relebactam

The SMART program provided isolates from the USA, which were used for testing the activity of imipenem combined with the newly developed, renally excreted carbapenemase inhibitor relebactam. Relebactam is structurally related to avibactam, differing only by the presence of an amide functional group bound to a piperidine ring (Figure 1). For KP, 99% and 96.1% of isolates were susceptible to imipenem-relebactam and imipenem alone, respectively, and 74.1% of imipenem-resistant isolates were rendered susceptible to the carbapenem by addition of relebactam (67). In vitro assays showed that the combination imipenem/relebactam decreased the MICs of KPC-producing KP compared to imipenem alone (MIC₅₀: 0.25/4 mg/L, MIC₉₀: 1/4 mg/L), but was not as effective against pathogens expressing class D enzymes (e.g., OXA-48 in KP) (68). To date, phase II clinical trials have reported that imipenem/relebactam is as effective as imipenem alone for treatment of complicated UTIs, including acute pyelonephritis (69).

Imipenem-relebactam is currently investigated in the frame of phase III clinical trials for the treatment of imipenem-resistant infections.

Vaborbactam

Vaborbactam (VB) is a boronic acid-based non-beta lactam beta-lactamase inhibitor, registered by FDA in 2017 for therapy of complicated urinary tract infections, combined with meropenem (2g/2g, intravenous q8h, to be adjusted in patients with renal impairment). Its chemical structure is not related to the ones of avibactam or relebactam (Figure 1).

Similar to meropenem, VB is renally excreted, and up to 60% of a dose is found unchanged in the urine within 24 hours. VB inhibits class A beta-lactamases (including KPC) and class C AmpC β -lactamases.

The meropenem/VB combination decreases the MICs of most resistant *Enterobacteriaceae* (2-fold to > 1024-fold decrease), though it appears that the addition of VB does not improve the activity of meropenem against *P. aeruginosa* (70).

Meropenem/VB is active against CREB isolates and

against *Enterobacteriaceae* showing multidrug-resistant and extensively drug-resistant phenotypes (MIC_{50/90}: 0.5/32, 0.03/1, and 0.5/32 mg/L, respectively), though this drug combination showed limited activity against isolates expressing metallo- β -lactamases (e.g., NDM-1, VIM) and oxacillinases (e.g., OXA-48, OXA-163) detected in Asia-Pacific and in certain European countries (71). The TANGO-1 phase III clinical trial has reported superiority of meropenem/VB over piperacillin/tazobactam for the treatment of complicated UTIs, including acute pyelonephritis (72). The TANGO-2 randomized, open-label phase III clinical trial of meropenem/VB versus "best available therapy" in patients with complicated urinary tract infections, bacteremia or pneumonia, was stopped early due to a benefit-risk ratio in favor of meropenem/VB (73).

Polymyxins

The polymyxin antibiotics colistin (poliomyxin E) and poliomyxin B have become a mainstay in the treatment of CREB infections. The EUCAST breakpoint for resistance is 2 mg/L. Recent studies resulted in the recommendation to prescribe high doses of the antibiotic (up to 10 million international units), divided into twice- or thrice-daily administrations (74). Importantly, combination therapy including colistin and rifampin was shown to be effective against colistin-resistant, KPC-producing KP (75). Though new polymyxin derivatives with decreased toxicity are under development, nephrotoxicity occurring in about 40% of cases and neurotoxicity are limiting factors to the extensive administration of colistin for treatment of CREB-induced infections. A possible limiting factor to the full exploitation of polymyxins for UTIs may also be the limited renal clearance of these drugs, though pharmacokinetic studies seem to confirm that the urinary recovery of colistin may be sufficient to attain concentrations above the MICs shown for example by XDR *P. aeruginosa* (76).

Aminoglycosides

Plazomicin is a novel aminoglycoside ("neoglycoside") antibiotic closely related to sisomicin and structurally recalling gentamicin. Its molecular structure was designed to be resistant against aminoglycoside-modifying enzymes, which are often expressed in CREB isolates (77). For example, in non-NDM-expressing CREB, the MICs of plazomicin ranged between > 0.5 and 2 mg/L, compared to 0.25/> 256 mg/L and 1/128 mg/L for gentamicin and amikacin, respectively (78).

Three-hundred multidrug resistant ESBL-producing and/or carbapenemase-producing *Enterobacteriaceae* isolates from Athens, Greece (a CREB endemic area), most of which were also resistant to previous generation aminoglycosides (e.g., MIC₅₀/MIC₉₀ to amikacin = 32/> 32), were tested for sensitivity to plazomicin. This novel aminoglycoside retained activity against all tested isolates of *K. pneumoniae*, *E. coli*, and *Enterobacter* spp., with MIC₅₀ and MIC₉₀ of 1 and 2 μ g/ml, respectively, irrespective of their multidrug-resistant phenotype (79). In strains of CREB resistant to gentamicin, tobramycin and amikacin, plazomicin exhibited an MIC range of 0.12-4 mg/L, with MIC₅₀ and MIC₉₀ values of 0.25 and 1 mg/L,

respectively. Interestingly, in CPKP isolates, synergy was observed when plazomicin was combined with meropenem, colistin or fosfomicin, whereas the combination with tigecycline resulted in indifference (80).

The safety and efficacy of plazomicin vs. levofloxacin in treating UTIs was assessed in a phase II comparative study, which ended in 2012 (81). Microbiological eradication in the plazomicin group (15 mg/kg) was achieved in 93.1% of the patients whose baseline urinary pathogens had a plazomicin MIC of 4 mg/L.

Microbiological eradication in the group receiving levofloxacin (750 mg) was achieved in 93.8% (15/16) of patients with levofloxacin-susceptible baseline urinary pathogens (MIC = 4 mg/L). The EPIC and CARE phase III trials, investigating the safety and efficacy of plazomicin in patients with complicated UTIs and other infections caused by gram-negative pathogens including CREB, have been concluded but not yet published in the form of journal articles.

Fosfomicin

Only case reports are available showing the efficacy of fosfomicin -administered in monotherapy for uncomplicated UTIs or combined with colistin against urosepsis-infections caused by *Enterobacteriaceae* expressing KPC, OXA-48 and NDM carbapenemases (82, 83). Case reports include accounts of successful combination therapy including dual carbapenem plus oral fosfomicin in UTIs caused by NDM-expressing *Enterobacteriaceae* (57). Such approach is preferable, as the rapid development of resistance during therapy is a major problem related to the usage of fosfomicin as single agent (84).

Intravenous formulations of fosfomicin allow administration of high doses of the drug (e.g., 4g q6h), if necessary (85). A small prospective case series including eleven critically ill ICU patients affected by CPKP bacteremia, UTI or pneumonia, was based on administration of intravenous fosfomicin (2-4g q6h) for about 14 days, combined with colistin (n = 6), gentamicin (n = 3) or piperacillin/tazobactam (n = 1), based on ascertained susceptibility. All-cause hospital mortality was 18.2%, and no infection relapse was observed in enrolled patients. Intravenous therapy with high-dose fosfomicin appeared to be well tolerated, without renal or liver function test abnormalities (86). Further large-scale studies are warranted to confirm these encouraging safety and efficacy results.

Oritavancin

Oritavancin is a semi-synthetic lipoglycopeptide, characterized by a threefold mechanism of action: (i) inhibition of the transglycosylation pathway in the bacterial wall synthesis, (ii) inhibition of the transpeptidation step by binding to the peptide bridging cell wall segments and (iii) cell wall disruption.

A recent *in vitro* subset study in the frame of a prevalence analysis performed on over 140.000 bloodstream isolates of Enterococci from Europe and the USA demonstrated that oritavancin was active against vancomycin-resistant *E. faecalis* (MIC_{50/90}, 0.25-0.5 mg/L), and showed MIC₅₀, MIC₉₀ and MIC₁₀₀ values of 0.03, 0.12 and 0.25 mg/L against VanA-positive *E. faecium*, respectively (87).

Oritavancin is renally and fecally excreted as unmodified molecule and may represent an interesting agent for treating complicated UTIs, though such approach would be off-label, as this drug is approved for the moment only for Gram-positive skin and skin structure infections.

Fluoroquinolones

Chen et al. have examined in detail how the pharmacokinetic-pharmacodynamic properties of fluoroquinolones could be exploited to design treatment strategies in areas with high rates of fluoroquinolone resistance (43). Levofloxacin appears to be a better option compared to ciprofloxacin, since the excretion of the former is by 87% renal (ciprofloxacin: 50%), and since efflux pumps like AcrAB, MdfA and NorE are more active on the latter (88). Thus, as the bactericidal activity of these agents is concentration-dependent, the achievement of high peak urine concentrations as a result of high-dose treatment with levofloxacin may be the key for eradicating pathogens showing intermediate FQ resistance levels. For example, a single 750 mg dose of levofloxacin achieves a mean urinary C_{max} of 620 mg/L, with prolonged post-antibiotic effect, compared to 340 mg/L attained by a standard 500 mg dose (89). *Chen et al.* also suggested that in areas with resistance uropathogen rates > 20%, high-dose levofloxacin might be considered as an option for UTIs caused by *E. coli* isolates showing a MIC ≤ 32 mg/L, after taking into consideration the potential adverse effects of such therapy (43).

The aac (6')-I gene product (AAC) confers to *Enterobacteriaceae* resistance to aminoglycoside antibiotics through acetylation of specific -NH₂ residues at the level of specific amino sugars. A mutated variant of AAC (aac (6')-Ib-cr) confers resistance to certain fluoroquinolones via acetylation of the (= NH) residue within the piperazine ring of drugs like ciprofloxacin or norfloxacin. This decreases by 4-fold the susceptibility of pathogens to such drugs (90) and increases by 16 times the fluoroquinolone mutant prevention concentration in *E. coli* (0.2 → 3.2 mg/L), thus facilitating the survival of target site mutants (91). Interestingly, FQs like levofloxacin have a methylated piperazine residue which is virtually protected from the action of AAC. Thus, if resistance rates caused by the AAC are present or suspected, levofloxacin, or other "protected" FQs like prulifloxacin or pefloxacin may be temptatively administered.

Glycylcyclines

Together with colistin, tigecycline is often the only agent to which CPKP is residually susceptible. However, the use of this agent for treatment of urological infections is significantly associated with subsequent development of resistance, as shown by *van Duin and coworkers* (OR, 6.13; 95% CI, 1.15-48.65) (92).

CONCLUSIONS

In conclusion, in the next decades the world will be facing a major medical emergency generated by the rapid spread of pathogens carrying resistance determinants of unprecedented power. All medical specialties will be affected by such spread, including foremostly urology.

As far as urinary tract diseases are concerned, we believe that the old definition of complicated vs uncomplicated infections should be modified, as any UTI involving carbapenemase-expressing uropathogens, or MDR/XDR uropathogens, should be considered and managed as complicated conditions.

Urgent containment measures must be put into effect, with priority given to those areas of the world characterized by climatic conditions favoring the seasonal outburst of infection epidemics, but also by negligent clinical practice, unprofessional pharmaceutical dispensing, as well as by poor patient compliance and education. Rigorous antibiotic stewardship and restriction of novel and old last resort agents to the sole hospital setting will also contribute to the containment of “superbug” epidemics.

Regretfully, major pharmaceutical companies have abandoned research for novel antibacterial agents due to the little financial reward ensured by drugs which can cure diseases in a very short time and at the same time may become rapidly obsolete due to the emerging of pathogen resistance.

Nevertheless, public or private research mainly aimed at discovering new therapies against Gram-negative organisms is of vital importance. Only the future will tell us who will prevail in the struggle for survival between humans and bacteria, and ultimately in the war between the immense resources of human ingenuity and the immense adaptability of genomes and species.

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