Comprehensive characterization of Escherichia coli isolated from urine samples of hospitalized patients in Rio de Janeiro, Brazil

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CHAPTER 7

SUMMARIZING DISCUSSION AND FUTURE PERSPECTIVES
Summarizing Discussion

Although, urinary tract infections (UTIs) can be caused by several microorganisms, extraintestinal pathogenic *E. coli* (ExPEC) is the main etiological agent causing both community- (CA) and hospital- (HA) acquired UTIs [1]. Whereas UTIs in outpatients are mostly self-limited and uncomplicated, they increase the morbidity and mortality of hospitalized patients and can progress to more complicated infections such as urosepsis [2]. The increased resistance levels of *E. coli* to antibiotics, including third generation cephalosporins and fluoroquinolones, largely reduces therapy options making such UTIs difficult to treat. Globally observed increase of resistance is associated with successful spread of specific *E. coli* clonal lineages, also called high-risk clones [3], [4]. Among such clones not only the enhanced resistance but also the presence of specific virulence factors is being observed. The characterization of *E. coli* focusing on virulence and resistance has important clinical and epidemiological implications.

Whole-genome sequencing (WGS) techniques have been used not only to identify resistant and virulent clones but also to monitor their evolution and global epidemiology [5], [6]. Such monitoring is important to identify newly emerging and spreading of known high-risk clones. The high-risk *E. coli* clones are very diverse and show a geographical-depending distribution [7], which makes the local investigation of these clones extremely important. In Brazil the resistance-rate of *E. coli* increased over the past few years [8], [9], but only a few studies have been performed so far, combining phenotypical and molecular characterization of UTI causing isolates. Therefore, the goal of the research described in this thesis was to characterise *E. coli* isolates obtained from urine samples of hospitalized patients in Rio de Janeiro looking at their fitness, virulence and drug resistance on various levels. Such studies contribute to a better understanding of the importance of the presence of high-risk clones causing UTIs inside Brazilian hospitals. In this final chapter, we deliberate on our findings and discuss future perspectives.

In this thesis, *E. coli* isolates obtained from urine samples of hospitalized patients collected between November-2015 and November-2016 were investigated. Based on the results of the work described in chapter 2 we observed an increase in antibiotic resistance rates compared to previous data available for Brazil. This included increased resistance to first choice antibiotics for treatment of uncomplicated UTIs, such as aminoglycosides, fluoroquinolones, trimethoprim and cephalosporins [10], [11]. Fortunately, the resistance rate to fosfomycin and nitrofurantoin was still low, but the resistance rate to aminoglycosides and fluoroquinolones was around 50%. As these antibiotics are commonly used to treat UTIs, they will not be effective in almost half of the patients if no proper diagnostics is performed. We also found that 28.9% of the isolates were ESBL-producers and that 4.6% were resistant to carbapenems. Surprisingly, most ESBL isolates had a CTX-M-15 type beta-lactamase, whereas previous studies reported a higher prevalence of CTX-M-2 and CTX-M-8 beta-lactamases in South-America [12], [13].
An association between antibiotic resistance and an increased global spread of high-risk lineages was reported before [7], [14], [15], and, therefore, we studied a possible association between the identified lineages and their antibiotic resistance and virulence profiles. Molecular typing of our isolates revealed that 30% of our isolates, including most of the multidrug resistant (MDR) and/or ESBL isolates, belonged to the high-risk \textit{E. coli} clones ST131, ST405 and ST648. Most sensitive isolates belonged to the successful lineages ST69, ST73 and ST10, that are not considered as high-risk clones. ST73 was described to be a highly virulent and emerging ESBL-producing lineage in other countries [20] and not surprisingly the most virulent isolates in our study belonged to this ST. Moreover, UTIs caused by ST69 and ST73 \textit{E. coli} were shown by previous studies to be responsible for community acquired infections in Brazil [16], [17]. Presence of such bacteria in our hospitalised patients may suggest a different dissemination of these clones among the community and hospitals in Brazil. Alternatively, a possible change in the predominance of successful clones within time may have occurred. Since there is no surveillance program in Brazil to monitor successful \textit{E. coli} clones and only few data is reported by research groups, it is impossible to make final conclusions.

Isolates of our study belonged to different phylogenetic groups, i.e., to A, B1, B2, D or F. Phylogenetic groups B2 and D are considered to be highly virulent in humans and are mostly associated with UTIs and blood-stream infections worldwide [18], [19]. Interestingly, isolates collected from UTIs in our study that belonged to the successful lineages ST73, ST131, ST648 all were classified as phylogenetic group B2. Most \textit{E. coli} isolates in our belonged to ST131. This lineage was first identified both in Canada and the UK in the 2000s [20], [21]. Since then it evolved and successfully spread worldwide [15] being associated with complicated UTIs and with primary sepsis. Characterization of this particular lineage was done in chapter 2, chapter 3 and chapter 5. The ST131 isolates can be classified according to their \textit{fimH} allele into \textit{fimH}30 (H30) and other \textit{fimH} types known to be less associated with resistance [22], [23]. H30 isolates resistant to fluoroquinolones are named H30-R and those resistant to fluoroquinolones and producing CTX-M-15 beta-lactamase are called H30-Rx. \textit{E. coli} can be further classified, based on the presence of virulence genes, into virotypes A to D. Most of our ST131 isolates were classified as viertype A or C. Virotipe C is known to be the most prevalent ST131 virotype in several countries, while virotipe A is more prevalent in Asia [24], [25]. Different from previous studies, in which virotipe C was associated with \textit{H}41 ST131 isolates[26], in our study virotipe C was associated with \textit{H}30 ST131 isolates. This discrepancy may be caused by many factors such as evolution over time, geographic location, antibiotic selective pressure or sample bias because of the small sample size of isolates from Brazil.

Although ST131 has been extensively studied, the mechanisms of emergence and dissemination of this clone is still not fully understood. A balance between the presence of specific virulence and resistance genes may be an important prerequisite. It allows bacteria to survive during antimicrobial treatment and at the same time to escape from the host’s immune response [27]– [29]. Indeed, virulence genes are essential for \textit{E. coli} pathogenesis and are mostly acquired through uptake of mobile genetic elements (MGEs), especially pathogenic islands (PAIs), discussed in chapter 3. PAIs are transmitted hori-
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zon tally and contain a block of virulence genes that can even be shared between species [30], [31]. Thus, MGEs are associated with increased virulence and enhanced resistance to different antibiotic classes [30], [32], [33]. Among successful lineages the presence of antimicrobial-resistance genes is associated with the acquisition of plasmids, particularly IncF plasmids. IncF-like plasmids are known to be involved in the acquisition of blaCTX-Ms resistance genes in ST131 isolates [34], [35].

So far there is not much data on MGEs available for Brazilian E. coli isolates. In chapter 3 the presence of MGEs such as plasmids, genomics islands (GIs), PAIs and phages in ST131 isolates was investigated and compared with their presence in other successful lineages. We found that MDR isolates, particularly ESBL-producing ones, predominant-ly contained IncF-type plasmids carrying several resistance genes. Typing of plasmids based on pMLST showed that types F1:A2:B20 and F2:A1:B- were more often found in H30-ST131 antibiotic resistance isolates, whiletype F29: A:-B10 was more frequently detected in susceptible H41/ST131 and ST69 isolates. The IncF plasmids in ST648 isolates were classified as F1:A1:B1. In theST131 and ST648 isolates, we also identified blocks of resistance genes organized between insertion sequences (IS26) and transposons (Tn3) known as resistance cassettes. They were present on plasmids, on phage-related chromosomal sequences and on the chromosome. These resistance cassettes often contained blaCTX-M15 (cephalosporin resistance) and aac(6’)-Ib-cr (fluoroquinolone resistance) genes and were not found in lineages not associated with an MDR or ESBL profile. Based on our results we could associate the high-risk clones and their resistant phenotype with the presence of IncF plasmids and resistance cassettes. This is in agreement with findings of others showing the importance of the acquisition of MGEs for the evolution of high-risk clones and that IncF plasmids are associated with the spread of the blaCTX-M15 and aac(6’)-Ib-cr resistance genes [36].

Among investigated ST131 isolates, different resistant cassettes were identified at different locations. Specifically, the ST131 isolates of virotype C H30-Rx carried two resistance cassettes of which one was located on a plasmid, similar to plasmid pEC958 of reference ST131 strain EC958 isolated in the UK [36]. In comparison to the reference plasmid, this resistance cassette did not contain the blaCTX-M15 gene. The second resistance cassette was found on the chromosome and did contain the blaCTX-M15 gene. Interestingly the ST131 isolates of virotype A H30-Rx contained the same resistance cassette including blaCTX-M15 than the one present on plasmid pEC958. In ST648 isolates, the resistance cassette was identified on phage-related chromosomal sequences. The observed different locations of resistance cassettes carrying the blaCTX-M15 gene is not rare and had been previously observed [26], [37], [38]. It had been suggested that the translocation of blaCTX-M15 into the chromosome is facilitated by IS26 elements and may be considered as a next step in the evolution of high-risk clones reducing the chance to lose it during replication. This may also be the reason of the presence of the gene in phage-related chromosomal sequences in the high-risk ST648 clone.
Similar to antibiotic resistance also virulence contributes to the success of a high-risk clone [39]. Therefore, we investigated the role of MGEs, such as PAIs, GIs, phages and plasmids and their association with virulence genes. Investigated ST131 isolates were compared to the reference strain EC958 that is known to be highly virulent. The genomic island GI-thrW, present in the reference strain EC958, appeared to be highly conserved among all ST131 isolates tested, but it was absent in isolates from all other lineages. The other GIs present in EC958, were present in some of our isolates belonging to the H30-ST131 sublineage. We also identified a new GI (GI-II) in our ST131 and ST69 isolates, which carried pap operon genes, several iron uptake system genes and toxins genes. In addition, highly diverse PAIs profiles were found even for isolates within the same lineage. Interestingly, the most similar PAI profile was found between virotype C H30Rx-ST131 and ST73 isolates. Most PAIs found in isolates from phylogenetic group B2 and D were absent in isolates from phylogenetic group A and B1, similar to previous observations [40], [41]. Surprisingly, the number of virulence genes associated with PAIs and plasmids, were also similar among ST131 and ST73. Although they showed a similar PAI distribution, their plasmids profiles are very different. Curiously, these isolates had a lower number of virulence genes associated with plasmids and PAIs than the ST69 and ST648 isolates in our study. Previous studies about the virulence of high-risk clones showed that ST131 isolates are associated with specific genes as intA, sat, iba, hyD, malX, ompT and truT [44], also identified in our ST131 isolates. Therefore, we hypothesized that the interplay between resistance and virulence for the ST131 lineage is more associated with the presence of specific genes than with the virulence score (based on the number of virulence genes). In addition, an increased resistance and virulence without an increase in fitness cost could be the reason for the success of the ST131 lineages. Studying MGEs is useful to investigate the evolution of these lineages and to identify potential emerging clones. Indeed, the acquisition of a plasmid by the recently emerging high-risk ST410 clone, thereby increasing their antibiotic resistance showed the importance of characterization of MGEs present in high-risk clones [45], [46].

Bacteriocins are protein-based toxins that can kill closely related species [42], [43] and are generally considered to be part of an anti-competitor strategy. Clearly, these molecules may contribute to the success of high-risk clones. In chapter 5 we investigated the presence of bacteriocins in successful lineages. Although bacteriocins encoding genes were found in 26 ST131 isolates, we only identified three isolates that were able to produce bacteriocins of which two belonged to the H22-ST131 sublineage and one to H30-ST131. In these isolates the bacteriocins genes were located on plasmids. Different from the highly resistant H30-Rx ST131 sublineage, the H22-ST131 isolates are more frequently foodborne uropathogens [44], [45]. In our study, the H22-ST131 isolates con-tained three plasmids: a colE1 plasmid, an IncI1 plasmid containing the colicin Ib and the resistance gene bla\textsubscript{CMY-2}, genes, and an IncF plasmid containing the colicin Ia, microcin V and several other virulence and resistance genes. These plasmids with a combination of bacteriocins, other virulence genes and antibiotic resistance genes are similar to plasmids identified in bacterial species present in poultry and fecal human samples[46], [47]. The ST131 H30-ST131 isolate that produced bacteriocins was susceptible to antibiotics and contained only two plasmids in which the bla\textsubscript{CMY-2} gene was absent.
analyses of the ST131 isolates revealed that they did not contain any known immunity protein. However, they showed a high resistant phenotype once they were exposed to bacteriocin producing ST131 isolates. A possible mechanism for the resistance to bacteriocins without having the immunity protein was recently proposed [48] and was dependent on the lipopolysaccharide in the outer membrane and minor environmental changes that could affect the sensitivity to bacteriocins. The diversity of bacteriocins genes identified among our ST131 isolates was considerably small, which may explain why they were susceptible to bacteriocins produced by non-ST131 isolates. In our study, we did not identify bacteriocin genes in ST69 and ST405 isolates belonging to phylogenetic group D. These results are slightly different from previous reports, in which it was described that isolates of phylogenetic group D did carry bacteriocins [49], [50].

Previous studies showed an association between the presence of virulence genes and bacteriocins in UPEC [51], [52]. Indeed, we found an association between the presence of bacteriocins and the presence of virulence genes that are part of the iron uptake system. The presence of bacteriocins in plasmids containing also resistance and/or virulence genes may increase the spread of those plasmids among ST131 isolates, particularly within the H30 and H30-Rx sublineages and can enhance the chances of other ST131 sublineages to emerge. Our results showed that the presence of plasmids containing bacteriocins and resistance genes did not come with a high fitness cost. In contrast, they seem to increase the bacteria’s adhesion to and invasion ability into urothelial cells, indicating that the presence of bacteriocins can be an advantage for survival and competition against other isolates in the gut thereby increasing the virulence of the bacteria. This advantage can lead to the successful spread of H22-CMY-2-producing ST131 isolates, which, therefore, should be monitored in the future. In addition, the presence of bacteriocin-producing isolates may be an explanation of the prolonged colonization of the gut by ST131 isolates, which was already described in nursing homes and other healthcare facilities in other countries [53], [54]. Unfortunately, no surveillance program that monitor the colonization of patients by ST131 E. coli is in place in Brazil and we were not able to study if this was also the case in Brazil.

One of the strategies that may improve the management of UTIs inside the hospitals is to combine the microbiological diagnostic results with the patient’s risk-factors as we discussed in chapter 6. Several factors can increase the risk to acquire UTIs inside the hospitals and/or the probability of therapy failure. We investigated the patients’ risk factors of having a UTI caused by an MDR or ESBL-producing E. coli. Additionally, as biofilms are related with catheter-associated UTIs and with persistent infections we decided to also assess risk factors for having a UTI caused by a biofilm-forming E. coli. In addition, we tried to identify possible differences in type, virulence and resistance determinants between community acquired (CA) UTIs, hospital acquired (HA) UTIs and asymptomatic bacteriuria (ABU) and tried to reveal a possible correlation between the presence of patients’ risk factors and having an infection caused by a high-risk E. coli clone with a specific virulence and/or resistance profile.
Unfortunately, we were only able to get the complete data of 63 out of 107 patients of which isolates were obtained. Obviously, this limited the statistical power of further analysis. Also, information about the severity of infections was not available for all patients but may have had an important impact on patient outcome. Even with these limitations, our results may help to get insights into patients’ risk factors and the outcome of UTIs and asymptomatic bacteriuria. In our study, most of the collected isolates were from female patients (73%). Most patients were older than 60 years having most frequently co-morbidities as neurologic and neoplastic diseases. Additional risks were previous hospitalization, surgical procedures and catheterization. Most patients suffered from a CA- or HA-UTIs, while just 11% presented ABUs. Overall, these results were similar to previous studies on UTIs [55], [56]. Overall, the distribution of virulence and resistance genes in bacteria isolated from patients with UTIs and ABU were very similar.

As mentioned above, the frequency of MDR \textit{E. coli} in this study was high and most were ESBL-producing, mostly CTX-M-15-producing. Also, fluoroquinolones resistant isolates were found. In this thesis, we assessed risk factors for having a UTI caused by MDR/ESBL \textit{E. coli}. In our study, diabetes, neurologic and neoplastic diseases and an age older than 60 years were considered to be a risk of having a UTI caused by MDR \textit{E. coli}, including high risk-clones. This association may be explained by the reduced immune response due to age or received treatments, reduced mobility leading to use of catheter or diapers, or by a high concentration of sugar in the urine of diabetic patients [57], [58]. Also the use of antibiotics are known to increase the risk of UTIs caused by MDR uropathogens [58]. As biofilm formation reduces the success of antibiotic treatments and is involved in developing chronic and recurrent infections leading to infected kidneys, septicemia and death, we also investigated the risk of a UTI caused by biofilm-forming \textit{E. coli}. We did not find such an association, which may be due to the fact that most of our isolates were able to form biofilms. Indeed, previous studies also showed that around 80% of \textit{E. coli} from urine samples are able to produce biofilm [59], [60].

We also found an association between complicated UTIs and high-risk isolates. These results may be explained by the overall increased risk on UTIs for this group of patients that were previously hospitalized or stayed in nursing facilities knowing to be host risk-factors for infections caused by high-risk clones as ST131[61]. When we studied the patients’ outcome, our results showed that the mortality rate is higher in patients suffering from UTIs caused by MDR and ESBL-producing \textit{E. coli}. Also, an association between infections caused by high-risk clones and a higher mortality rate was revealed. However, this may be the result of the association between high-risk clones and having an MDR/ESBL profile. Another explanation for the higher mortality rate could be the combination of reduced antibiotic options for treatment and the increased susceptibility of these patient for infections due to comorbidities and the presence of other risk factors.

In this study we identified the presence of high-risk clones in hospitalized patients in Brazil. This, in combination with empirical therapy, may increase antibiotic resistance rates. Currently, fosfomycin is the first-choice drug in the treatment of uncomplicated
UTIs and it has been considered as a therapeutic option for complicated UTIs and infections caused by MDR E. coli [62], [63]. Fosfomycin resistance rates are low [64] and in our study below 3%. However, heteroresistance to fosfomycin may be an underestimated problem, as it is often not detected in routine diagnostics and is associated with therapeutic failure and recurrent UTIs [65]. In chapter 4, the genetic mechanisms that lead to the observed heteroresistant phenotype were studied. We found a higher frequency of fosfomycin heteroresistant isolates (9%) than described previously [66]. Please note, however, that comparing results from different studies is difficult as both the definition of heteroresistance and the methods to determine it are not standardized [65], [67].

Heteroresistance has been associated with mutations in genes encoding fosfomycin transporters (gfpT and uhpT), genes responsible for the regulation and expression of these transporters and in the gene encoding the fosfomycin target protein MurA [68][69]. We found different molecular mechanisms that may lead to a heteroresistant profile, which may be partially explained by the fact that our isolates were from different hospitals and belonged to different clonal groups and thereby evolved independently. Our results showed that at least in four of our heteroresistant isolates, a reduction in fosfomycin uptake was related with the heteroresistant profile. This could be due to the loss in function of the fosfomycin transporters or by mutations in genes encoding the regulatory proteins that control the expression of fosfomycin transporters. Our WGS data indicated deleterious mutations in gfpT and uhpT genes affecting the function of the transporter in some but not all of our isolates. In some other fosfomycin heteroresistant isolates, we identified, yet unknown, mutations in fosfomycin transporter genes and in genes responsible for the regulation and expression of these transporters. No mutations were identified in the murA gene itself. Our results indicate that there is no common genetic basis for the heteroresistance profile, and we were not able to clarify the mechanisms leading to fosfomycin heteroresistance in all studied isolates. Therefore, we used a transcriptomic approach to reveal differences in the expression levels of genes between fosfomycin resistant subpopulations and our fosfomycin heteroresistant isolates. In general, the fosfomycin resistant subpopulations showed an overexpression of the murA gene. In addition, several other genes, including virulence and SOS system genes, were over-or under expressed in the fosfomycin resistant subpopulations. The changes in expression levels of genes that are not directly related with the uptake or activity of fosfomycin may be a survival mechanism of the bacteria during environmental stress or can be caused by the heteroresistance profile itself.

Differently from the high fitness cost that come with mutations in the transporters and regulatory genes, the overexpression of the murA gene has been described as an antibiotic resistant strategy with low fitness cost [68], [70]. Therefore, we also investigated the fitness of the heteroresistant isolates and their resistant subpopulations. Our results showed that the mutations of genes associated with the fosfomycin uptake or an overexpression of the murA gene did not lead to high fitness cost in our isolates. These results are contradictory to previous studies in which it was hypothesized that the reason for rarely observed heteroresistance profile in vivo is due to the high fitness cost of the mutations leading to this profile [66]. However, mutations in the transporter
genes, leading to fosfomycin resistance without increasing the fitness cost, has been described for *S. aureus* and *P. aeruginosa* [71], [72]. We also studied if the heteroresistance profile affected the fosfomycin efficiency against biofilms. We showed that the fosfomycin can reduce the biofilm biomass of heteroresistant isolates but resulted only for half of these isolates in a significant biofilm viability reduction. These results indicate that a heteroresistant profile can affect the efficiency of fosfomycin against biofilms. This is particularly relevant for UTIs, as biofilms, as already mentioned, are linked with catheter associated UTIs, persistence and recurrence of UTIs [73]–[75]. Although this study has been performed in vitro and the impact of these results on patient outcomes still needs to be evaluated, the presence of mutations leading to fosfomycin heteroresistance in clones that are already highly resistant without affecting bacterial fitness is a huge medical concern. It also stresses the need of a better management system for antibiotic use, especially in countries such as Brazil which already suffer from the consequences of high antibiotic resistant levels. In addition, treating MDR *E. coli* with fosfomycin needs to be reconsidered to avoid introducing even more limited treatment options.

**Future Perspectives**

There is a lack of information on antibiotic resistance prevalence of *E. coli* in hospitals at the national level in Brazil. The few research reports available point out an increase of infections caused by antibiotic resistant *E. coli* in the past few years [9], [76], [77]. This increase may be associated with the emergence of high-risk clones and makes the treatment of UTIs more challenging. Therefore, it is key to improve the management of UTIs. This includes the use of strategies as antibiotic stewardship to both deaccelerate the increase of antibiotic resistance and to improve treatment. In Brazil this may be achieved through (i) the reinforcement of laws that would limit the sale of antibiotics, (ii) reduction of antibiotic use without prescription, (iii) education of the Brazilian population to reduce the unnecessary abuse of antibiotic consumption, (iv) and to determine and implement national guidelines for antibiotic use inside public and private hospitals [78]. These actions should be applied by both public health authorities and/or the (local) governments.

In addition, optimized molecular diagnostics and antibiotic susceptibility testing of the isolated bacteria in combination with an improved patient management system recording the patients history is key for reducing unnecessary and wrongly chosen antibiotic therapy [79]. For example, recurrent and/or chronic UTIs, caused by an ESBL-producing high-risk *E. coli*, can be better treated with carbapenems, whereas these should not be used for treatment of uncomplicated UTIs and/or UTIs caused by non-MDR bacteria. In this way, resistance rates to antibiotics such as nitrofurantoin, fosfomycin and carbapenems may [80], [81] remain low and effective to treat patients with more severe infections caused by MDR bacteria. Improved diagnostics and surveillance of UTIs may be achieved by introduction of novel molecular techniques, such as next generation sequencing (NGS). These technologies are already used in routine microbiology in some countries and the most essential.
information of the pathogen can be retrieved from the generated sequencing data. In fact, nowadays NGS starts to become essential for getting detailed insight into the epidemiology, evolution and identification of (new) high-risk clones. It helps to monitor spread of pathogens on local, national and international levels. Furthermore, the sequencing information is being used to support antibiotic stewardship. The implementation of NGS in routine diagnostics faces several challenges, as operational costs, validation and quality control issues, turnaround time, bioinformatics analyses and standardization of typing schemes and cut-off values [82], [83]. This may be partially solved by setting up a collaboration with partners who implemented this technology already in their routine microbiology laboratory. However, Brazil also faces political and economic crises, which make it even more challenging to implement these technologies in a standardized way.

Brazil used to be part of a global surveillance program between 2008-2013 that focused on identification of carbapenemase-producing E. coli ST131 isolates [26]. Unfortunately, only a small number of isolates were collected. Therefore, there is no well-defined data on prevalence of high-risk clones circulating in the hospitals in Brazil available. Moreover, the data collected so far are limited and may be biased as most studies obtained data from the same (south east or south) regions of the country [84], [85]. A future surveillance program that include all the regions of Brazil would be essential to provide an overview and give hint on epidemiology, prevalence and evolution of (newly emerging) high-risk E. coli clones in the whole country. This may be achieved by collecting information from all hospitals across the country and by collaboration of health care institutes with universities and other research institutes that are able to provide the laboratory facilities and/or perform the data analyses.

In summary, to improve the management of UTIs inside hospitals in Brazil, three steps need to be considered: further implementation and improvement of patient management systems, and of antibiotic-and diagnostic-stewardship programs. This will increase the knowledge on the epidemiology, and resistance and virulence characteristics of high-risk E. coli clones in Brazil, which on its turn may help to prevent the further spread of these bacteria and, more importantly, better protect patients against difficult to treat UTIs.
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Chapter 7


