Spread/Outbreak of multidrug-resistant *Klebsiella pneumoniae* in tertiary hospitals

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Multidrug-resistant *Klebsiella pneumoniae* is one of the most frequent opportunistic pathogens to cause nosocomial outbreaks in tertiary hospitals. The reason is that *K. pneumoniae* can be spread quickly from the gastrointestinal tract of the patients and further on by the hands of caretakers or inventory to colonize other patients, and thereby lead to nosocomial clonal outbreaks. In 2007 there was a major clonal outbreak of multidrug-resistant CTX-M-15 producing *K. pneumoniae* in Denmark, since then, the University Hospital of Copenhagen has been monitoring the multidrug-resistant *K. pneumoniae* clones in circulation using ribotyping. There are three main clonal ribotypes at this hospital. Even though the clonal outbreak from 2007 should now be under control, it is still the most frequent ribotype of isolated *K. pneumoniae.* This may indicate that certain multidrug-resistant *K. pneumoniae* strains have the potential to survive and stay in circulation in the hospital environment despite infection control measures.

Keywords Nosocomial; clonal outbreak; spread; *Klebsiella pneumoniae*; multidrug-resistant

1. Introduction

In the last decade infections caused by multidrug-resistant microorganisms have become a major threat for the human health. [1, 2] Especially nosocomial infections when hospitalized give problems with antibiotics susceptibility, because the hospital environment gives a constant selective pressure that favours resistance. Nosocomial infections are also one of the leading causes of mortality and morbidity in hospitals, and thus longer hospital stays and higher expenses. [3-6]

Nosocomial infections are often caused by multidrug-resistant Gram-negative microorganisms. [4, 7] One of these Gram-negative bacteria is *Klebsiella pneumoniae*, which is naturally found in the gut microbiota of both humans and animals. Because *K. pneumoniae* is a part of the normal gut microbiota, the development of drug-resistance can take place undetected, and studies have shown that *K. pneumoniae* is quick to develop resistance. [8-16] This gives *K. pneumoniae* the selective advantage and the ability to survive in environments where antibiotics are widely used, such as hospitals.

K. pneumoniae have also been isolated from various hospital environment sites such as polluted medical equipment, sink drains and handles, bar soaps and cleaning equipment, where there source is believed be contamination. [4-5, 8, 17-19] These findings were made during several investigations to determine possible reservoirs for cross infection sites. Our observations support these findings as we have found *K. pneumoniae* on handles and floors but also in the sewage outlet from University Hospital of Copenhagen (UHC). *K. pneumoniae* has also been isolated from environment sites where there is no obvious contamination; this could be soils, surface water and water used in the industry. [8] The habitat for *K. pneumoniae* is plentiful, which also means that the reservoirs for *K. pneumoniae* to survive and cause infections are just as plentiful.

This plentifulness of places where *K. pneumoniae* can survive is one of the reasons that *K. pneumoniae* is quickly to spread and cause infections. Another reason is the transfer from the gastrointestinal tract of the patients and further on by the hands of caretakers or inventory to colonize and infect other patients.Both can lead to nosocomial clonal outbreaks. [5, 18, 20]

Nosocomial infections caused by *K. pneumoniae* often strike patients having underlying diseases who therefore are immunocompromised. *K. pneumoniae* nosocomial infections can cause a long range of complications, but the six most frequent types of infections are urinary tract infection, pneumonia, septicaemia, wound infections, neonatal septicaemia and nosocomial infections in intensive care unit patients. [5, 11-12, 17, 21-24]

The purpose of this article is to compare results from the department of Infection Control UHC to the literature regarding multidrug-resistant *K. pneumoniae*. First a short review of the resistance-development in *K. pneumoniae* will be given.

2. Resistance of Klebsiella pneumoniae

Since the discovery of the antibiotics and the commercial use of these began, the resistance have followed the rate of the consumption and the development of new antibiotics. [24-25] In table 1 is listed when the resistance to the different antibiotic classes have been observed in *K. pneumoniae*. Today the multidrug-resistance in *K. pneumoniae* is often caused by the enzyme extented spectrum beta-lactamases (ESBL), which was registered for the first time in Germany in 1983. [3, 20, 24, 26] ESBL are derivatives of common beta-lactamases that have undergone one or more amino acid

substitutions near the active site of the enzyme. These alterations increase the affinity and hydrolytic ability of extended-spectrum cephalosporins and monobactams. [27]

For many years the most efficient antibiotic treatment against ESBL-producing *K. pneumoniae* have been the antibiotic class carbapenem, but now there has been a significant increase of carbapenem-resistant *K. pneumoniae*. [28] The enzyme carbapenemase exhibit activity against a wide range of beta-lactams, this includes penicillins, old and new cephalosporins, monobactams and carbapenems. [28] Most often both the ESBL and the carbapenemase are mediated by plasmids, often these plasmids also carry genes encoding resistance to other drug classes. [23, 28-29, 30-36] But studies have shown that loss of outer membrane protein also contribute to the resistance against carbapenem. [28, 36] It is these transferable plasmids that enable *K. pneumoniae* to rapidly gain antibiotic resistance. [13, 23]

Table 1 Development of drug resistant in *Klebsiella pneumoniae.*

Year	Drug class	Reference
1960´s	Most penicillin's	18, 37
1970´s	Aminoglycoside	10-11, 13-14
	Polypeptids	18, 38
1980´s	Cephalosporins	3, 20
	Monobactams	3, 39
	Quinolones	40
1990´s	Carbapenem	33-36

The level of resistance for *K. pneumoniae* varies much between countries, with the highest resistance found in the Latin America and the lowest in Asia Pacific, Scandinavia and the United Kingdom. The resistance level seems to be connected to the early and present use of antibiotics. [25, 24, 41-43]

3. *Klebsiella pneumoniae* at University Hospital of Copenhagen

One of the keystones in preventing spread and outbreak of harmful microorganisms is surveillance of the infection rate; at UHC this is performed by the Department of Infection Control. Since 2006 we have used ribotyping as a monitoring instrument for multidrug-resistant *K. pneumoniae.* The reason for using ribotyping is that the method is quick compared to the time consumption of Pulsed Field Gel Electrophoresis (PFGE), which is a well established method for typing *K. pneumoniae.*

In the period 2006-2012 we have ribotyped 873 multidrug-resistant *K. pneumoniae* isolates, and the majority (67.1%) of these isolates form three main clonal ribotypes (111-S6, 233-S5 and 237-S5), the ribotype pattern is shown in figure 1.

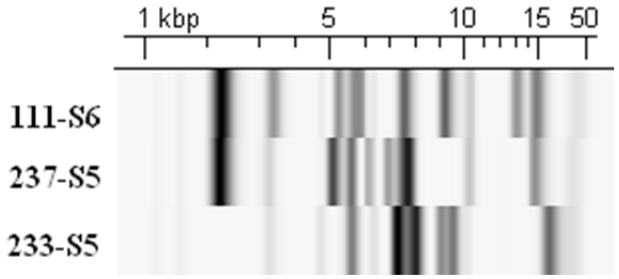

Fig. 1 The ribotype pattern for the three main types of *Klebsiella pneumoniae* isolated from patients hospitalized at University Hospital of Copenhagen.

The ribotype patterns show that ribotype 111-S6 and 237-S5 are more related to each other than to ribotype 233-S5. This is a fair conclusion since the ribotype method distinguish the *K. pneumoniae* isolates based on the difference in 5S, 16S and 23S ribosomal DNA. The ribotype that contains most isolates is 237-S5 with 430 (49.3%) *K. pneumoniae* isolates and none of those were isolated before 2007. The ribotype 111-S6 and 233-S5 have 89 (10.2%) and 63 (7.2%) *K. pneumoniae* isolates, respectively. The distribution of *K. pneumoniae* isolates between year 2006 and 2012 belonging to the three ribotypes is shown in figure 2.

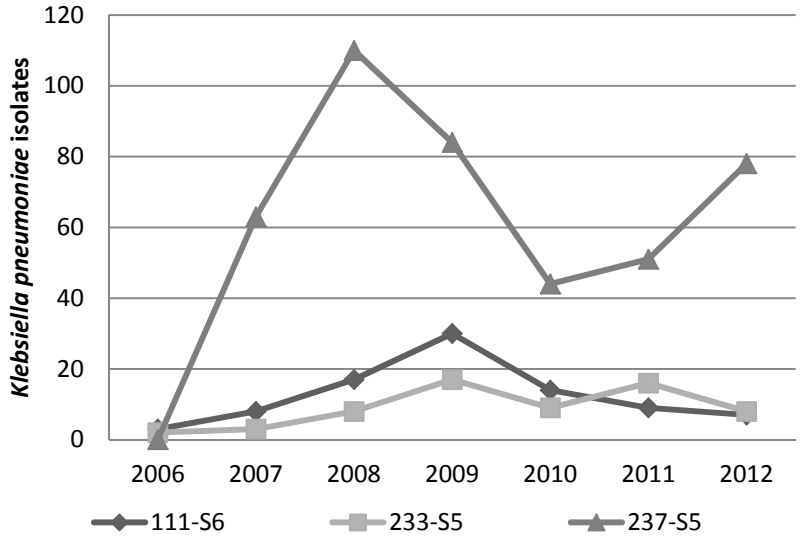

Fig. 2 The number of isolated *Klebsiella pneumoniae* between year 2006 and 2012 for the three most abounded ribotypes (111-S6, 233-S5 and 237-S5) at University Hospital of Copenhagen.

At UHC we consider *K. pneumoniae* multidrug-resistant if the isolate is resistant to at least two of the following antibiotics: Cefuroxime, Gentamicin and Ciprofloxacin. Table 2 shows the percentage of resistance to the indicator antibiotics for the *K. pneumoniae* isolates which are ribotyped. The numbers clarify that there is some common resistance pattern for the multidrug-resistant *K. pneumoniae* isolated at UHC, because there is a high number of isolates resistant to ciprofloxacin and cefuroxime for all three ribotypes.

Table 2 The resistance of the multidrug-resistant *K. pneumoniae* which are ribotyped at the University Hospital of Copenhagen.

Ribotype	Percentage of resistance		
	Ciprofloxacin	Gentamicin	Cefuroxime
111-S6	97	4.4	97.7
237-S5	98.6	77.6	100
233-S5	93.6	80.8	93.6

3.1. Infection Control

At the UHC we follow the official Danish recommendations for controlling multidrug-resistance and highly pathogenic microorganisms immediately after identification. The measures are isolation of positive patients, barrier precautions (e.g. gowns and gloves for staff entering the patient's room) and extra environmental disinfection of the room. Furthermore, the former roommate(s) must be screened to identify possible transmission of the microorganisms. If the screening is positive, the patients on the entire ward will be screened, to find positive carriers to avoid further spreading in the hospital, because many patients of at UHC are treated across specialities. If the screening shows an outbreak, the ward will be closed for intake of patients.

Our normal infection control measurement prescribe that the staff performs hand hygiene before and after caretaking of patients and change gowns if they are visibly dirty.

4. Discussion

Nosocomial infections caused by multidrug-resistant *K. pneumoniae* are a rising problem worldwide.A search in the literature revealed that *K. pneumoniae* is quick to develop resistance to antibiotics that are frequently used. Clonal spread of *K. pneumoniae* can accrue undetected because the time interval between detection of the isolates can be long. This may result in spreading of *K. pneumoniae* clones to other hospitals because of patients transferring. [2-3, 7, 10, 12, 15, 18, 22, 44-46] Already in the 1960´s an outbreak caused by multidrug-resistant *K. Pneumoniae was described*, where the primary isolation sites were urinary tract and chest. [18-19, 48]

Of the three main ribotypes of multidrug-resistant *K. pneumoniae* which often cause nosocomial infection at UHC, we only know the origin of one – ribotype 237-S5. The ribotype 237-S5 is identical to the ribotype of a multidrug-resistant CTX-M-15-producing *K. pneumoniae* which caused a major clonal outbreak in year 2007 in Denmark. [11, 46-47] The origin of the outbreak was Hillerød Hospital from where UHC has received patients for dialysis. We believe

that this is the transfer-route for the spreading of ribotype 237-S5 to our hospital. Although the outbreak from 2007 should be under control, ribotype 237-S5 is still the most frequent type for the multidrug-resistant *K. pneumoniae* causing nosocomial infections at UHC. A study regarding the most prevalent types at the hospitals in Zealand of Denmark also shows that the Hillerød-clone is the most abounded *K. pneumoniae* to cause nosocomial infections in Denmark. [36, 37] The Hillerød-clone has a Multi Locus Sequence typing (MLST) ST15, and this CTX-M-5-producing *K. pneumoniae* clone type is also common in hospitals in Hungary, which may be the origin of the Hillerød-clone. [49]

The year before the outbreak only 16 multidrug-resistant *K. pneumoniae* isolates causing nosocomial infections at UHC were ribotyped, where none was identical to ribotype 237-S5. In year 2007 where the outbreak began we experienced an explosive increase in the isolation rate of multidrug-resistant *K. pneumoniae* (114 isolates),more than 700% increase. Since the outbreak we have not been able to reduce the number of isolated multidrug-resistant *K. pneumoniae* to the level before the outbreak, despite the additional monitoring and increased focus on hygiene.

The CTX-M-15 enzyme found in the multidrug-resistant *K. pneumoniae* clone which caused the Danish outbreak, has been found in multidrug-resistant *K. pneumoniae* clones causing outbreaks around the world; US, France, Spain, Hungary, Tunisia, Asia, India and Sweden. [26, 49, 50-55] CTX-M-15 has also been shown to be the most abounded resistance gene in hospitals over many years. [26, 50, 56] Because of the dissemination of CTX-M-producing strains in hospital environments worldwide it is not possible to remove the enzyme from the environment in spite of better infection control at our hospital. Because CTX-M enzymes are so widely spread some argue that adapting the current detection methods so they survey these enzymes could give a better control of the spread. [60]

We do not know the origin of the multidrug-resistant *K. pneumoniae* clones belonging to the ribotype 111-S6 and 233-S5, but we have found the ribotype 111-S6 in the sewage from UHC, which may imply that ribotype 111-S6 can be spread to the community, thereby also spreading the multidrug-resistance.

To have a polyclonal population of multidrug-resistant *K. pneumoniae* present in the hospital environment is not uncommon. [15, 26, 47] The tendency seen in our hospital has not been described before, where a single clone is able to survive and become the dominating clone for so many years after the clone has been introduced to the environment from an outbreak. Most often, the surveys are performed over long time periods only looking into the resistance development of *K. pneumoniae* and for short periods looking into the clonal correlation. [5, 23-25, 31, 41, 49, 52-53, 61] It is not clear which is the best way for monitoring the presence of multidrug-resistant *K. pneumoniae* in the hospital environment. A retrospective study performed by Coque T M, et. al., 2002 where they investigated the resistance genes and the clonal relationship for the ESBL-producing *K. pneumoniae* isolated between the years 1989 – 2000, revealed that most ESBL-producing *K. pneumoniae* clones were not able to be maintained in the hospital setting, while the genes were. The observations made by Coque T M, et. al., 2002 are supported by other shorter studies. [14, 26] With that knowledge the best surveillance of the spread of multidrug-resistant *K. pneumoniae* must be to monitor both the clonal relationship and the genes present in the isolates.

The UHC is a tertiary hospital, and in many areas a hospital just like ordinary hospitals, but patients to tertiary hospitals are transferred from other hospitals and are often very sick when hospitalized. Because patients are transferring from another hospital we often know if the patients are infected with multidrug-resistant *K. pneumoniae*, and therefore infected patients are isolated on admission. It has been shown that isolation of infected patients on admission is not enough to prevent the spreading of multidrug-resistant *K. pneumoniae* in tertiary hospitals. [12, 32] Furthermore, asymptomatic carriers seem to be the main way of spreading outbreak clones to other wards, but also the hygiene of the caretakers are important for preventing the spread. [15, 32, 50, 57] Many studies have shown that emphasis on the necessity of good hygiene to the caretakers significantly reduces the spread. [5, 11, 15, 32]

In conclusion, our investigation gives some indications that certain multidrug-resistant *K. pneumoniae* clonal strains may have the potential to survive and stay in circulation in the hospital environment despite infection control measures. In the future we need to be more aware of the asymptomatic patients, because it seems they play an important role in controlling the spread of multidrug-resistant *K. pneumoniae* at the tertiary hospitals. Furthermore, if the survival of multidrug-resistance is based more on the plasmids circulating in the hospital environment, new methods need to be taken into consideration in order to detect outbreaks.

It would also make sense to monitor polyclonal populations of multidrug-resistant *K. pneumoniae*, to ensure that no single clone suddenly becomes dominant, which would indicate a beginning epidemic state.

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