

The emergence of multidrug-resistant *Klebsiella pneumoniae* of international clones ST13, ST16, ST35, ST48 and ST101 in a teaching hospital in the Paris region

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SUMMARY

Despite infection control measures, an important increase in the extended-spectrum β -lactamase (ESBL)-producing *Klebsiella pneumoniae* incidence density occurred in our hospital from 2006 onwards. This study, focusing on the 2005–2007 period, was performed in an attempt to explain this increase. ESBLs were characterized, isolates were typed by ERIC2-PCR, and sequence type (ST) of clustered isolates was determined. Temporal-spatial relationships of patients were analysed to assess possible cross-contamination. Of the 74 ESBL-producing isolates, 30 (40%) were detected at admission, 53 (71.5%) produced CTX-M enzymes, 40 displayed unique ERIC2-PCR profiles and 34 were assigned into six clusters: ST16 ($n=21$), ST101, ST48, ST35, ST13, and ST436. Relationships were identified in 22 of the 34 patients harbouring clustered isolates. This study highlights the complex epidemiology of ESBL-producing *K. pneumoniae* in the mid-2000s with potential cross-contamination for only 30% of the 74 patients in our hospital, and the emergence of clones that are currently spreading worldwide.

Key words: Antibiotic resistance, hospital-acquired (nosocomial) infections, infectious disease epidemiology, *Klebsiella*, molecular epidemiology.

INTRODUCTION

Klebsiella pneumoniae is a major nosocomial pathogen. Isolates producing extended-spectrum β -lactamase (ESBL) appeared in the early 1980s and were involved in numerous outbreaks, especially in intensive-care units (ICUs) [1–3]. While hygiene

measures permitted the control of ESBL-producing *K. pneumoniae* nosocomial infections in the 1990s, ESBL-producing *Escherichia coli* emerged in the early 2000s, not only in hospitals but also in the community [1]. Whereas *K. pneumoniae* isolates were in the main producers of TEM and SHV derivatives, *E. coli* isolates were shown to produce mainly CTX-M enzymes [1]. In the mid-2000s, public health concern increased due not only to the worldwide dissemination of CTX-M-producing *E. coli*, but also to the local

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emergence of carbapenamase-producing *K. pneumoniae* [4].

β -lactamase gene dissemination results from a combination of plasmid dissemination in genetically distinct strains and clonal spread of plasmid-harboring resistant strains [1, 4, 5]. Indeed, within *E. coli* species, clone sequence type (ST)131 isolates producing in particular CTX-M-15, have been identified globally [6]. Similarly, within *K. pneumoniae*, clones ST11 and ST258 grouped into clonal group (CG) 258 [7], and clone ST14 grouped into CG15 [7], have been involved in several outbreaks on different continents [5, 7].

In our hospital, measures to control transmission of ESBL-producing organisms have been implemented since late 2004. These are based essentially on (i) notification of ESBL-producing Enterobacteriaceae carriers (identified from clinical samples or rectal swabs performed at admission for patients with previous history of hospitalization or at admission, and once a week for patients being hospitalized in ICUs), and (ii) implementation of precaution barriers.

In spite of these measures, the annual incidence density of ESBL-producing *K. pneumoniae*, [expressed as the annual number of patients carrying an ESBL-producing *K. pneumoniae* per 1000 hospitalization days (HD)], evolved as follows: 0.07 in 2005, 0.21 in 2006, 0.32 in 2007, 0.31 in 2008 and 0.27 in 2009 (our unpublished data).

In order to better characterize the determinants of the sharp increase in incidence density observed in 2006, we undertook a retrospective study focusing on the molecular features of isolates and epidemiological links of patients during the 2005–2007 period.

METHODS

Hospital setting

Beaujon Hospital is a 461-bed teaching hospital of Assistance Publique-Hôpitaux de Paris (AP-HP) that comprises seven surgical wards, seven medical wards, two ICUs [ICU(A) and ICU(B)], a maternity unit, and an emergency unit. The hospital specializes in chronic digestive diseases ($n = 161$ beds).

Bacterial isolates

The first ESBL-producing *K. pneumoniae* isolate recovered from faecal or clinical samples in each patient hospitalized between January 2005 and December

2007 was included in the study (one isolate per patient). Faecal isolates had been selected on Drigalski agar plates containing 1 mg/l cefotaxime. Isolate identification was performed by the API System (bioMérieux, France) and ESBL production was assessed by using the double disk synergy test [2].

ESBL characterization

Genes encoding ESBL enzymes (*bla*_{CTX-M}, *bla*_{TEM}, *bla*_{SHV} types) were characterized by polymerase chain reaction (PCR) and DNA sequencing with specific primers, as described previously [8].

ERIC2-PCR typing

Isolates were typed using the ERIC2-PCR method as described previously [6]. Briefly, 1 mM of primer (5'-AAG TAA GTG ACT GGG GTG AGC G-3') and amplification conditions including 44 cycles and a 36 °C hybridization temperature were used. PCR products were further submitted to 100 V electrophoresis for 1.5 h in 1.5% agarose gels.

Two isolates were considered to be genetically related (cluster) when they exhibited a strictly identical ERIC2-PCR profile.

Epidemiological data

Hospitalization dates and wards were obtained from the hospital database. ESBL-producing *K. pneumoniae* isolated after 48 h of hospitalization were deemed hospital acquired whereas isolates recovered within the 48 h following admission were considered as imported. The imported isolates were further grouped as healthcare associated, when patients had undergone hospitalization during the previous 3 months, or as community acquired, when patients had no contact with any healthcare setting during the previous 3 months.

As recovery of genetically related isolates (i.e. from the same ERIC2-PCR cluster) in different patients could suggest cross-transmission, we looked for temporal-spatial relationships of patients (patients being hospitalized during overlapping days in the same ward) over the study period.

Multi-locus sequence typing (MLST)

The international MLST scheme (www.pasteur.fr/mlst) [9] was used to determine the ST of clustered

Table 1. Distribution of the carriers of an ESBL-positive *Klebsiella pneumoniae* isolate according to ward and sample type, and number of patients admitted with this isolate from 2005 to 2007

| Ward/sample | Number of patients (%) | | | |
|---------------------|------------------------|---------|-----------|-----------|
| | 2005 | 2006 | 2007 | Total |
| Total | 10 | 26 | 38 | 74 |
| Imported | 5 (50) | 9 (35) | 16 (42) | 30 (40.5) |
| Rectal | 2 | 2 | 8 | 12 |
| Clinical | 3 | 7 | 8 | 18 |
| Hospital acquired | 5 (50) | 17 (65) | 22 (58) | 44 (59.5) |
| Rectal | 3 | 12 | 12 | 27 |
| Clinical | 2 | 5 | 10 | 17 |
| Intensive-care unit | 5 | 9 | 16 | 30 |
| Imported | 2 (40) | 0 (0) | 2 (12.5) | 4 (13) |
| Rectal | 1 | 0 | 1 | 2 |
| Clinical | 1 | 0 | 1 | 2 |
| Hospital acquired | 3 (60) | 9 (100) | 14 (87.5) | 26 (87) |
| Rectal | 3 | 7 | 11 | 21 |
| Clinical | 0 | 2 | 3 | 5 |
| Medicine | 2 | 12 | 11 | 25 |
| Imported | 0 (0) | 6 (50) | 6 (54.5) | 12 (48) |
| Rectal | 0 | 2 | 4 | 6 |
| Clinical | 0 | 4 | 2 | 6 |
| Hospital acquired | 2 (100) | 6 (50) | 5 (45.5) | 13 (52) |
| Rectal | 0 | 4 | 0 | 4 |
| Clinical | 2 | 2 | 5 | 9 |
| Surgery | 2 | 2 | 5 | 9 |
| Imported | 2 (100) | 0 (0) | 2 (40) | 4 (44) |
| Rectal | 1 | 0 | 2 | 3 |
| Clinical | 1 | 0 | 0 | 1 |
| Hospital acquired | 0 (0) | 2 (100) | 3 (60) | 5 (66) |
| Rectal | 0 | 1 | 1 | 2 |
| Clinical | 0 | 1 | 2 | 3 |
| Others | 1 | 3 | 6 | 10 |
| Imported | 1 (0) | 3 (100) | 6 (100) | 10 (100) |
| Rectal | 0 | 0 | 1 | 1 |
| Clinical | 1 | 3 | 5 | 9 |

ESBL, Extended-spectrum β -lactamase.

isolates including, for each cluster, representative isolates from patients without a temporal and spatial link.

Statistical analysis

Categorical variables were compared by Fisher's exact test while Student's *t* test was used to compare continuous data. All statistical analyses were performed by Epi Info (CDC, USA). A *P* value of ≤ 0.05 was considered as statistically significant.

Table 2. ESBL types of *Klebsiella pneumoniae* isolates over the study period

| ESBL type | Number (%) of isolates | | | |
|-----------|------------------------|----------|----------|-----------|
| | 2005 | 2006 | 2007 | Total |
| TEM | 5 (50) | 3 (12) | 3 (8) | 11 (15) |
| 3 | 4 | 2 | 1 | 7 |
| 12 | 0 | 1 | 1 | 2 |
| 21 | 1 | 0 | 0 | 1 |
| 167 | 0 | 0 | 1 | 1 |
| SHV | 1 (10) | 4 (15) | 5 (13) | 10 (13.5) |
| 2 | 0 | 1 | 3 | 4 |
| 12 | 1 | 3 | 2 | 6 |
| CTX-M | 4 (40) | 19 (73) | 30 (79) | 53 (71.5) |
| 3 | 1 | 0 | 0 | 1 |
| 15 | 2 | 19 | 27 | 48 |
| 14 | 0 | 0 | 2 | 2 |
| 27 | 0 | 0 | 1 | 1 |
| 99 | 1 | 0 | 0 | 1 |
| Total | 10 (100) | 26 (100) | 38 (100) | 74 (100) |

ESBL, Extended-spectrum β -lactamase.

RESULTS

Bacterial isolates

From January 2005 to December 2007, 74 patients were detected as carriers (from rectal swab or clinical sample) of ESBL-producing *K. pneumoniae* (10 in 2005, 26 in 2006, 38 in 2007) (Table 1). Rectal swabs provided 39 isolates whereas 35 were obtained from clinical samples [urine (16), blood culture (6), drainage liquids (6), surgical sites (2), abscess (1), ascites (1), catheter (1), genital (1) and neonatal (1)]. Whatever the year, isolates recovered from rectal swabs numbered about the same as those recovered from clinical samples (Table 1).

ESBL characterization

CTX-M-type enzymes were identified in 53 (71.5%) of the 74 isolates whereas TEM and SHV derivatives were identified in 15% and 13.5%, respectively (Table 2). These three types of ESBLs were present each year. CTX-M-15, SHV-12 and TEM-3 were the most commonly identified ESBLs (Table 2). The number of CTX-M-producing isolates (especially CTX-M-15) increased over the 3 years, whereas the number of TEM and SHV ESBL-producing isolates remained stable (Table 2). A new variant of CTX-M was identified from a rectal swab isolate obtained

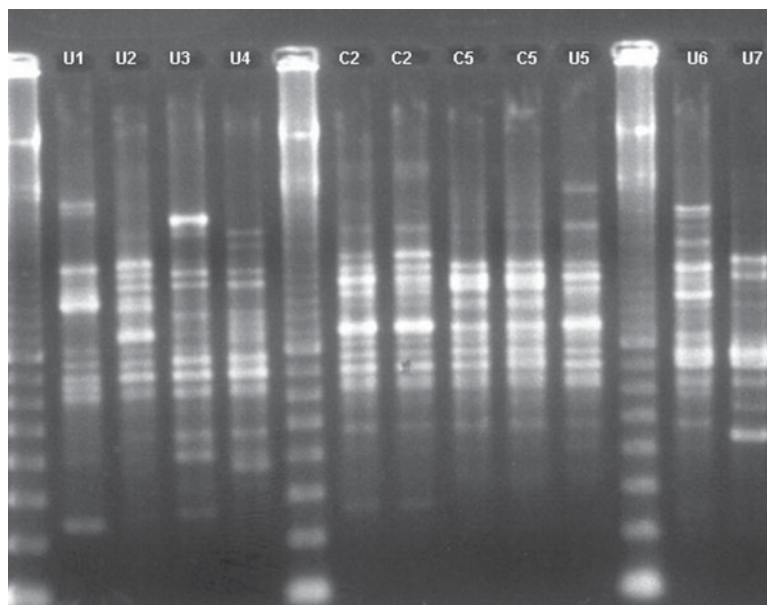


Fig. 1. Examples of ERIC2-PCR profiles from ESBL-producing *Klebsiella pneumoniae*. Seven unique profiles (U1, U2, U3, U4, U6, U7), and two representative isolates of cluster 2 (C2) and cluster 5 (C5) are presented. Lanes 1, 6 and 12 contain molecular-weight marker.

from a patient hospitalized in ICU(A) in January 2005. This enzyme showed serine at position 167 (as in CTX-M-19) and arginine at position 274 (as in CTX-M-24). This enzyme was designated CTX-M-99 and its sequence has been registered in public sequence databases under accession number HM803271.

Epidemiological data

The 74 patients colonized or infected with ESBL-producing *K. pneumoniae* were hospitalized in 15 of the 17 clinical wards of our hospital. Many (40%) of these patients were in ICUs, whereas 34% and 12% were in medical and surgical wards, respectively (Table 1). For 44 (60%) patients, the isolate was hospital acquired. The remaining 30 (40%) patients carried the ESBL-producing *K. pneumoniae* isolate at admission. For 18 of them, the isolate was healthcare associated and for 12, it was community acquired. The proportion of carriers at admission was not significantly different each year ($P=0.6$). However, carriers at admission were significantly less frequent in ICUs than in the other clinical wards (13% vs. 64%, $P<10^{-4}$) (Table 1).

ERIC2-PCR typing

The 74 isolates showed 46 ERIC2-PCR profiles (Fig. 1) corresponding to 40 unique profiles and six

clusters of 2–21 isolates. Of the 30 imported isolates, 23 displayed unique ERIC2-PCR profiles whereas seven were classified into clusters. Of the 44 hospital-acquired isolates, 17 displayed unique ERIC2-PCR profiles and 27 belonged to clusters. Unique profiles were significantly more frequent in imported isolates than in hospital-acquired isolates ($P=0.002$), consistent with the increased risk of cross-transmission within the hospital.

Temporal-spatial relationships in patients and STs of genetically related strains

One strain producing SHV-2 was transmitted in 2007 by a mother to her newborn during delivery. Both isolates which grouped into cluster 1 and were of ST35 were considered as imported (Table 3).

Cluster 2 comprised 21 isolates, of which one was imported by a patient who had stayed in ICU(A) during a previous hospitalization at the same time as another cluster 2 carrier. The 21 isolates were recovered from 15 rectal swabs and six clinical samples in 2006 and 2007. They all produced CTX-M-15 and displayed ST16 according to the MLST method applied to eight representative isolates. The 21 patients were hospitalized in seven different wards but 14 of these patients, including the index case, were cared for in ICU(A) when their ESBL-producing isolate was detected. Hospital stay overlaps were observed in

Table 3. *Epidemiology and sequence type of six ERIC2-PCR-defined clusters of ESBL-producing Klebsiella pneumoniae*

| Cluster | No. of isolates | Year of isolation | No. of imported isolates | No. of wards | ESBL type | Sequence Type (ST) | No. of patients with temporal-spatial link |
|---------|-----------------|-------------------|--------------------------|----------------|-----------|--------------------|--|
| 1 | 2 | 2007 | 2 | 1 ^a | SHV-2 | ST35 | 2 |
| 2 | 21 | 2006–2007 | 1 | 7 ^b | CTX-M-15 | ST16 | 14 |
| 3 | 4 | 2006–2007 | 2 | 1 ^c | CTX-M-15 | ST101 | 3 |
| 4 | 2 | 2006–2007 | 1 | 1 ^d | CTX-M-15 | ST436 | 0 |
| 5 | 2 | 2006–2007 | 1 | 2 ^e | TEM-3 | ST13 | 0 |
| 6 | 3 | 2005 | 1 | 2 ^f | TEM-3 | ST48 | 3 |

ESBL, Extended-spectrum β -lactamase.

^a Maternity.

^b ICU(A), ICU(B), neurosurgery, digestive system surgery, colorectal surgery, gastroenterology and internal medicine.

^c Hepatology.

^d Internal medicine.

^e Gastroenterology and neurosurgery.

^f ICU(B) and digestive system surgery.

12 of the 14 patients hospitalized in ICU(A), clearly suggesting cross-contamination in this ward. Three other patients with cluster 2 isolates, hospitalized in three different wards (neurosurgery, gastroenterology, internal medicine) at the time of detection of being an ESBL carrier, had previously stayed in ICU(A). However, for two of these patients, ICU(A) hospitalization occurred during a period with no cluster 2 carrier detection. Of the four remaining patients, one was hospitalized in a surgery ward at the same time as a patient known to have contracted the epidemic strain in ICU(A) some weeks ago, and the three others were hospitalized in wards where other patients with cluster 2 had never stayed [ICU(B) and digestive system surgery] or had stayed 3 months previously (internal medicine). Overall, of the 21 patients carrying cluster 2, 14 had both temporal and spatial relationships whereas two patients had neither temporal nor spatial relationships with other carriers. For the five remaining patients, there was a spatial link (same ward) with other cluster 2 carriers, but without temporal overlap. As the study was retrospective, it was not possible to look for environmental strains of cluster 2.

Similar features were observed for the other clusters (Table 3): temporal-spatial relationships between three of the four patients harbouring cluster 3, spatial but not temporal relationships between the two patients harbouring cluster 4, no temporal-spatial relationship between the two patients harbouring cluster 5, and temporal-spatial relationships between the three patients harbouring cluster 6. The STs determined for two representative isolates of clusters 3,

4 and 5, and one of cluster 6 were ST101, ST436, ST13 and ST48, respectively.

Overall, for the 34 patients carrying clustered isolates, hospital stay overlaps with other patients harbouring the same cluster were documented in 22 cases (Table 3).

DISCUSSION

Based on 57 participating hospitals, the rate of *K. pneumoniae* blood isolates resistant to extended-spectrum cephalosporins increased from 4% in 2005 to 10% in 2007 in France (EARS-net, <http://www.rivm.nl.earss/>). Such an evolution has also been observed in other parts of the world [10–12]. The multiplication by 4.4 of the incidence density of ESBL-producing *K. pneumoniae* in our hospital between 2005 and 2007 is, thus, in accordance with the evolution observed elsewhere. Therefore, we were interested in determining which factors could explain this increase in spite of the implementation of infection control measures.

Compared with the epidemiological features of ESBL-producing *K. pneumoniae* identified in the 1980s, the present study provides noticeable differences. In the past, ESBL-producing *K. pneumoniae* were essentially observed in the hospital setting, as is currently the case for carbapenamase-producing *K. pneumoniae* [4]. In contrast, between 2005 and 2007, 40% of strains were imported in our hospital and 16% were apparently community acquired. Our results are in accord with those from recent studies from Spain [13] and Canada [11]. In the past,

ESBL-producing *K. pneumoniae* isolates were essentially found in ICUs [1, 3], whereas in our study the 74 patients carrying ESBL-producing *K. pneumoniae* were hospitalized in 15 of the 17 wards. We identified, as largely documented, nosocomial cross-transmission especially in ICUs, but the number of patients involved in cross-contamination in ICUs as well as in the other wards was low (22/74, 30%). We found that the number of *K. pneumoniae* isolates producing CTX-M increased each year and that this enzyme was predominant, whereas the number of isolates producing TEM and SHV enzymes was stable. A shift from TEM and SHV to CTX-M type enzymes in *K. pneumoniae* has recently been reported in different studies [11, 12, 14]. Nevertheless, in the present study we identified two enzymes (TEM-3, SHV-2) that were commonly produced by *K. pneumoniae* in the past [3].

Hence, our findings concur to suggest that a change occurred in the epidemiology of ESBL-producing *K. pneumoniae* in the mid-2000s, similar to the shift observed for *E. coli* in the early 2000s [1] when this species became the predominant CTX-M-producing Enterobacteriaceae.

The 74 ESBL-producing *K. pneumoniae* isolates displayed a polyclonal structure. However, this polyclonal structure was dominated by a few clusters over the study period, one of which (cluster 2, ST16) was highly predominant as it included 21 persons. We showed that this predominance was not related to cross-transmission in all of the cases. Three hypotheses could explain the recurrence of clusters in the same ward: (i) contamination from patients whose colonization was not detected, (ii) persistence of the strain in the hospital environment [15, 16], and (iii) the repeated introduction into the hospital of globally disseminated clones. The latter phenomenon has been well described for *E. coli* ST131 [17].

ST16, the dominant clone in our study, has already been described in Paris in the early 2000s in a non-ESBL producer [9]. Next, ST16 has been shown to be involved in outbreaks of CTX-M-15-producing *K. pneumoniae* in Sweden [18] and Denmark [19, 20], respectively, in 2005 and 2008, and reported as a KPC producer in Brazil in 2008 [21, 22]. It should be noted that ST16 is a single locus variant (SLV) of ST17, which has been reported as an ESBL producer in Spain (2008) [23], Denmark (2008) [19], Canada (2002) [11] and Asia (2008) [12], as a DHA-1 producer in Spain (2007) [24], and as a KPC producer in Greece (2009) [25]. Therefore, it is possible that isolates of ST16 and ST17 are part of a clone disseminated

worldwide. Previously [7], we defined clonal groups as groups including a chosen ST (central genotype), its SLVs (first circle SLVs), and the SLVs of the first circle SLVs (second circle SLVs). However, current MLST diversity (based on the 971 STs in the *K. pneumoniae* database at www.pasteur.fr/mlst) does not allow defining a meaningful clonal group that would encompass ST16 and ST17, as the application of our previous definition to ST16 or ST17 as central genotypes would include a large proportion of all *K. pneumoniae* STs. The difficulty in defining clonal groups in *K. pneumoniae* is related to the high rate of recombination between STs [26].

ST101, identified in this study in four patients, is another widespread clone harbouring various β -lactamases: CTX-M-15 in 2003 in Tunisia [27]; both CTX-M-15 and OXA-48 in 2009 in France, from a patient transferred from Tunisia [28], and in Barcelona where it caused a hospital outbreak [29]; OXA-48 in Israel from a Palestinian patient in 2011 [30]; SHV-12 in 2006 in Eastern European countries [31]; CTX-M-15 in 2008 in Asia [12]; and, finally, KPC in 2007 in the USA [32] and in 2009 in Brazil [21].

ST48, producing TEM-3 and identified in three patients with temporal-spatial relationships, has already been described as producing SHV-12 in 2003 in Tunisia [27] and CTX-M-15 in 2006 and 2008 in Korea [33,34].

ST35 producing SHV-2 was recovered in a mother and her newborn. ST35 has also been reported in Tunisia (SHV-12 in 2002) [27], in Spain (CTX-M-15 in 2008) [23], and in Denmark (without ESBL in 2008) [19].

ST13 identified in two patients in our study without an epidemiological link, was reported in Curaçao during an ESBL-producing *K. pneumoniae* outbreak in 2005 [9] and in Spain during a DHA-1-producing *K. pneumoniae* outbreak in 2007 [24]. Moreover, ST13 is a SLV of ST327 that has been reported to harbour CTX-M-15 in Spain [35] and KPC in Israel [36] and Brazil [22].

The last cluster, producing CTX-M-15 and identified in this study in two patients without a link, displayed ST436, which is unrelated to any another ST and, to our knowledge, has not been reported previously.

In conclusion, this study shows the complex epidemiology of ESBL-producing *K. pneumoniae* over the 2005–2007 period, during when a sharp increase in ESBL-producing *K. pneumoniae* incidence density was observed in our hospital and elsewhere. On the one

hand, the polyclonal structure of ESBL-producing *K. pneumoniae* detected over the 2005–2007 period, together with the analysis of the temporal-spatial relationships of patients, strongly suggest that cross-contamination is not the major cause of the increased incidence density. On the other hand, we observed that a few clonal groups isolated from both epidemiologically and non-epidemiologically linked patients, corresponded in fact to clones that have been reported in other countries, occasionally producing distinct enzymes. These observations suggest that these clones might have emerged as early as 2006, as observed in our hospital, and had diffused widely since then as was documented for ST258/KPC-2 [22]. Alternately, the fact that distinct enzymes are harboured by these clones in different countries suggests independent β -lactamase gene acquisition, perhaps because these clones are frequent in human commensal flora and subsequently frequently exposed to antibiotics, as described for ST131 *E. coli* [37]. Better knowledge of the distribution of these STs/clonal groups in digestive tract carriage and other sources will be important to understand better the global epidemiology of ESBL-producing *K. pneumoniae*.

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DECLARATION OF INTEREST

None.

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