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Specific Distribution within the *Enterobacter cloacae* Complex of Strains Isolated from Infected Orthopedic Implants▽

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Bacteria belonging to the *Enterobacter* genus are frequently isolated from clinical samples but are unusual causative agents of orthopedic implant infections. Twelve genetic clusters (clusters I to XII) and one sequence crowd (sequence crowd *xiii*) can be distinguished within the *Enterobacter cloacae* nomenspecies on the basis of *hsp60* sequence analysis, and until now, none of these clusters could be specifically associated with a disease. In order to investigate if specific genetic clusters would be involved in infections of orthopedic material, two series of bacterial clinical isolates identified as *E. cloacae* by routine phenotypic identification methods were collected either from infected orthopedic implants ($n = 21$) or from randomly selected samples of diverse anatomical origins (control; $n = 52$). Analysis of the *hsp60* gene showed that genetic clusters III, VI, and VIII were the most frequent genetic clusters detected in the control group, whereas cluster III was poorly represented among the orthopedic implant isolates ($P = 0.006$). On the other hand, *E. hormaechei* (clusters VI and VIII), but not cluster III, is predominantly associated with infections of orthopedic implants and, more specifically, with infected material in the hip ($P = 0.019$). These results support the hypothesis that, among the isolates within the *E. cloacae* complex, *E. hormaechei* and *hsp60* gene sequencing-based cluster III are involved in pathogenesis in different ways and highlight the need for more accurate routine *Enterobacter* identification methods.

Prosthetic joint infection (PJI) is, after aseptic loosening, the second most frequent complication of prosthetic joint replacement. Improvements in surgical techniques and the prevention of infection have lowered the risk of infection for primary hip or knee replacement to less than 1 and 2%, respectively, but the incidence of infection can increase 10-fold in the case of surgical revision (30). The population at risk for PJI continues to steadily increase, with an estimated 1 million arthroplasties being carried out worldwide each year (2), and the socioeconomic burden could become considerable (14–16). The average cost of combined medical and surgical treatment of an infected joint prosthesis is estimated to be $30,000, with important discomfort and substantial economic consequences for the patient (4). Besides joint prosthetic implants, other implantable devices such as screws and internal fixation devices are also at risk for infection in a wide range of clinical situations. Gram-positive cocci are the most frequent pathogens encountered, but members of the *Enterobacteriaceae* family can also be involved (29). Among these, *Enterobacter* species are major nosocomial pathogens often found in intensive care settings, and their role in PJI remains to be documented.

Bacteria of the *Enterobacter* genus are widely encountered in nature. These microorganisms are saprophytic in the environment and commensal in the enteric flora since they are found in soil and sewage, as well as in the human gastrointestinal tract (17, 22). The taxonomy of the *Enterobacter* genus has been iteratively updated (8–12, 19). Several species are described in this taxon (*Enterobacter aerogenes*, *E. amnigenus*, *E. cancerogenus*, *E. cowanii*, *E. gergoviae*, *E. intermedius*, *E. pyrinus*), as is a genetic complex, referred to as the "*E. cloacae* complex," in which other species have been identified (*E. asburiae*, *E. kobei*, *E. ludwigii*, *E. hormaechei*, *E. nimipressuralis*, and *E. cloacae*). *E. sakazakii* strains, which belong to several genomospecies, were recently reassigned to the newly proposed genus *Cronobacter* (12).

Enterobacter species can act as pathogens, and the *E. cloacae* complex, commonly referred to as "*E. cloacae*," represents the *Enterobacter* group most frequently encountered in human clinical samples. Although identification as "*E. cloacae*" is routinely performed by phenotypic methods in clinical laboratories, the accurate identification of isolates within this taxon is difficult. Analysis of the 16S rRNA gene is widely used for bacterial identification, but it is poorly discriminatory for closely related members of the *Enterobacteriaceae* family and, more specifically, for members of the *Enterobacter* genus (27).

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Other targets for use for the molecular identification of isolates within the *Enterobacter* genus have been described, such as the *oriC* locus (23), *gyrB* (6), *rpoB* (8, 21), and *hsp60* (8). Sequence analysis of a fragment of the *hsp60* gene showed that the *E. cloacae* nomenspecies could be divided into 12 genetic clusters (clusters I to XII) and one sequence crowd (sequence crowd *xiii*). Specific names could be attributed to some of the genetic clusters: *E. asburiae* (cluster I) (9), *E. kobei* (cluster II) (9), *E. ludwigii* (cluster V) (11), *E. nimipressuralis* (cluster X) (8), *E. cloacae* subsp. *cloacae* (cluster XI) (9), and *E. cloacae* subsp. *dissolvens* (cluster XII) (9). Although the name *E. hormaechei* was sometimes used as a generic name for strains belonging to different *hsp60* gene sequencing-based clusters (20), clusters VI, VII, and VIII together formally constitute the *E. hormaechei* species, which has three subspecies: *E. hormaechei* subsp. *oharae* (cluster VI), *E. hormaechei* subsp. *hormaechei* (cluster VII), and *E. hormaechei* subsp. *steigerwallti* (cluster VIII) (10). Species names were not attributed to clusters III, IV, and IX and to sequence crowd *xiii*, although at least cluster III is of significant clinical importance (8, 26).

The degree of genomic diversity within the *E. cloacae* complex was recently reassessed by more global genotypic methods. Multilocus sequence analysis (MLSA) identifies seven clusters within the *E. cloacae* complex, and each of these corresponds to one or more *hsp60* gene sequencing-based genetic clusters. Microarray-based comparative genomic hybridization analysis (CGH) showed two genetically distinct clades (21). Most strains associated with clinical disease belong to the youngest CGH-based clade, to which strains of *hsp60* gene sequencing-based clusters III, VI, and VIII also belong. The second, and older, CGH-based clade comprises heterogeneous strains, some of which are associated with commensalism.

Single-locus-based molecular methods, as well as global approaches, such as MLSA or CGH, suggested that some genetic clusters are more prone to cause infection, although no specific epidemiological association could be demonstrated (8, 21). The repeated occurrence in our clinical laboratory of orthopedic implant infections due to *E. cloacae* isolates prompted us to further investigate these strains. We analyzed strains isolated from infected orthopedic devices and compared them to randomly selected strains isolated from clinical specimens taken from diverse anatomical sites. Analysis of the *hsp60* gene showed that among the genetic clusters of the *Enterobacter cloacae* complex, clusters VI and VIII, but not cluster III, are predominantly associated with infections of orthopedic implants and, more specifically, with hip implants. These data support the hypothesis that genetic clusters of the *E. cloacae* complex are involved in pathogenesis in different ways and highlight the need for more accurate routine methods for the identification of *Enterobacter* species.

MATERIALS AND METHODS

Bacterial strains and epidemiological data. For the study group, strains were collected from three large academic hospitals in the Paris, France, area (Ambroise Paré Hospital, Raymond Poincaré Hospital, and Cochin Hospital, AP-HP) involved in the management of osteoarticular infectious conditions. In each hospital, all bacterial strains isolated from orthopedic device-related surgical samples are systematically and prospectively collected and stored, irrespective of the bacterial species, the anatomical site of isolation, or the type of infection. For the study group, the collections were submitted to identical screening criteria (1999 to 2006 for Ambroise Paré Hospital, 2000 to 2006 for Raymond Poincaré Hospital, 2005 to 2007 for Cochin Hospital) for the selection of strains belonging to the *E. cloacae* complex that were responsible for the infection of the orthopedic implants. The following criteria were applied for inclusion of the strains in the study: (i) identification of the infecting organism as *E. cloacae* with routine phenotypic identification systems (the API 20E or the Vitek 2 system; BioMerieux, Marcy l'Etoile, France), (ii) clinical evidence of infection of the orthopedic implant in an adult patient, (iii) isolation of the *E. cloacae* strain from surgical samples collected at the point of contact between surrounding tissue and the implanted material (or from the implant itself) at the time of surgical treatment, and (iv) involvement of the *E. cloacae* strain as a probable causative agent for the infection and clinical management as such. Isolation of an *E. cloacae* strain from a wound, sinus tract, or drainage was not sufficient to ascertain its involvement in the implant infection; and such strains were not included in the study. One strain per patient was included, and in the case of the isolation of multiple isolates, the most clinically relevant isolate (i.e., from the implant itself rather than from the surrounding tissue) was used. Twenty-one strains were selected and are referred to as strains isolated from orthopedic implanted material (Table 1). Since different types of implants were involved, only the anatomical site of infection was considered for data analysis. All selected strains recovered from storage at −80°C were viable for subculture. Personal data (age, sex, site of infection, mono- or polymicrobial infection) as well as the hospital and the date of isolation were anonymously collected for the purposes of this study.

For the control group (Table 2), 52 randomly selected clinical strains routinely identified as *E. cloacae* in the clinical laboratory at the academic Cochin Hospital by use of the API 20E or the Vitek 2 identification system were prospectively collected from clinical samples taken for diagnostic purposes from adult patients during the year 2006; environmental isolates were excluded. Thus, the control group was representative of *E. cloacae* strains from an adult population routinely identified in a clinical laboratory. One isolate was included per patient, and the strains collected were registered on the basis of the anatomical site of isolation, as follows: skin and soft tissue ($n = 14$), upper and lower respiratory tract ($n = 15$), urine ($n = 12$), joint or bone (in the absence of infected material; $n = 3$), intravascular catheter ($n = 2$), blood ($n = 2$), and gastrointestinal suppuration with exclusion of feces ($n = 4$). Personal data (age, sex, site of isolation) were anonymously collected for the purpose of this study.

Identification methods. Bacterial DNA from bacterial colonies grown overnight at 37°C on 5% horse blood agar plates was prepared for PCR analysis by using the Instagene nucleic acid purification method (Bio-Rad, Marnes la Coquette, France). Partial sequencing of the *hsp60* gene was performed by a previously described protocol (8). Briefly, oligonucleotide primers Hsp60-F (5′-GGTAGAAGAAGGCGTGGTTGC-3′) and Hsp60-R (5′-ATGCATTCGGTGGTGATCATCAG-3′) were used for genomic amplification of a 341-bp fragment of the *hsp60* gene. A negative control containing all reagents except the target DNA (which was replaced by H_2O) was included in each series. PCR was performed on a GeneAmp PCR system 9700 apparatus (Applied Biosystems) for 30 cycles by using the following conditions: 30 s at 94°C for denaturation, 30 s at 57°C for annealing, and 60 s at 72°C for elongation. Both strands of the purified amplified DNA fragment were sequenced by the BigDye Terminator cycle sequencing protocol with the same primers used for the PCR. Chromatograms of the complementary strands obtained with an ABI 313 apparatus (Applied Biosystems) were assembled by using the VectorNTi suite of programs (Invitrogen Corp.). A 272-bp fragment of the *hsp60* gene was obtained for the 73 strains, and the sequence of the fragment was compared to reference sequences from strains previously described in taxonomic studies (8) by using the Clustal W algorithm (www.align.genome.jp). Sequence comparisons were exported as an unrooted neighbor-joining tree with proportional branch lengths.

Statistical analysis. Epidemiological associations were analyzed by use of the Fisher exact test and the corresponding two-tailed *P* value.

Nucleotide sequence accession numbers. The sequences of the following type strains were retrieved from the GenBank database (the information in parentheses is the strain designation, GenBank accession number): *E. asburiae* (ATCC 35953, AJ417141), *E. kobei* (ATCC BAA260, AJ567899), *E. cloacae* subsp. *dissolvens* (ATCC 23373, AJ417143) (9), *E. ludwigii* (EN-119, AJ417114) (11), *E. hormaechei* subsp. *oharae* (EN-314, AJ543782), *E. hormaechei* subsp. *hormaechei* (ATCC 49162, AJ417108), *E. hormaechei* subsp. *steigerwaltii* (CIP108489, AJ543908) (10), *E. nimipressuralis* (ATCC 9912, AJ567900), *E. cancerogenus* (ATCC 33241, AJ567895), *E. amnigenus* (ATCC 3072, AJ567894), *E. cowanii* (ATCC 107300T, AJ567896), *E. gergoviae* (ATCC 33028, AJ567897), *E. pyrinus* (ATCC 49851, AJ567901), *C. sakazaki* (ATCC 29544, AJ567902) (12), and *E. aerogenes* (AB008141). For *E. cloacae* subsp. *cloacae*, no *hsp60* sequence is available for strain ATCC 13047, but one can be found under GenBank accession number AJ417142 (strain ATCC 13049),

TABLE 1. Characteristics of strains isolated from orthopedic implanted material[a]

Strain	Sex	Age (yr)	Hospital	Yr of isolation	*hsp60*-based genetic cluster	Site of infection	Form of isolation
P1	F	70	CCH	2006	I	Elbow	With associated flora
P2	F	37	CCH	2006	III	Tibia	With associated flora
P3	F	23	APR	2006	III	Tibia	Pure
P4	F	79	APR	1999	V	Ankle	Pure
P5	F	61	APR	2006	V	Knee	Pure
P6	M	35	CCH	2007	XI	Ankle	With associated flora
P7	F	57	RPC	2005	VI	Knee	With associated flora
P8	F	60	APR	2005	VI	Knee	Pure
P9	M	33	RPC	2006	VI	Hip	Pure
P10	M	79	RPC	2004	VI	Hip	With associated flora
P11	F	53	APR	1999	VI	Hip	Pure
P12	M	36	CCH	2006	VIII	Femur	Pure
P13	F	72	RPC	2006	VIII	Knee	With associated flora
P14	F	26	CCH	2007	VIII	Hip	With associated flora
P15	M	42	CCH	2006	VIII	Hip	Pure
P16	M	63	RPC	2002	VIII	Hip	Pure
P17	F	66	RPC	2000	VIII	Hip	Pure
P18	M	56	APR	2001	VIII	Hip	Pure
P19	M	77	APR	2005	VIII	Hip	With associated flora
P20	M	25	CCH	2005	VIII	Tibia	Pure
P21	M	23	APR	2005	VIII	Tibia	With associated flora

[a] The ratio of males (M) to females (F) was 0.9. The mean age ± standard deviation was 51 ± 20 years. CCH, Cochin Hospital; APR, Ambroise Paré Hospital; RPC, Raymond Poincaré Hospital.

which was used in another study (13). The GenBank accession numbers for previously described strains (8) are listed in the Fig. 1 legend. The GenBank accession numbers for the clinical strains described in this work are FJ595719 to FJ595791.

RESULTS

Distribution of strains isolated from infected orthopedic implants within the genetic clusters of the *Enterobacter cloacae* complex. *Enterobacter* is an uncommon causative agent of orthopedic implant infections. Investigation of the databases of three large academic orthopedic surgical centers in the Paris, France, area yielded 21 cases of orthopedic device infections due to *E. cloacae* on the basis of routine phenotypic identification methods (Table 1). The infections were found to be polymicrobial in 9 cases (43%), whereas isolates phenotypically identified as *E. cloacae* were found as the sole causative agent in 12 patients (57%). Partial sequencing of the *hsp60* gene allowed the identification of all isolates as part of one of the clusters that form the *E. cloacae* complex (Fig. 1). Some genetic clusters appeared to account for most of the cases: 15 strains (71%) belonged to the *E. hormaechei* species (clusters VI and VIII), 2 (9%) belonged to cluster III, and 2 (9%) belonged to cluster V (*E. ludwigii*). *E. cloacae* subsp. *cloacae* and *E. asburiae* (clusters XI and I, respectively) were found only once; and clusters II (*E. kobei*), IV, VII (*E. hormaechei* subsp. *hormaechei*), IX, X (*E. nimipressuralis*), and XII (*E. cloacae* subsp. *dissolvens*) were absent from among the isolates in the study group.

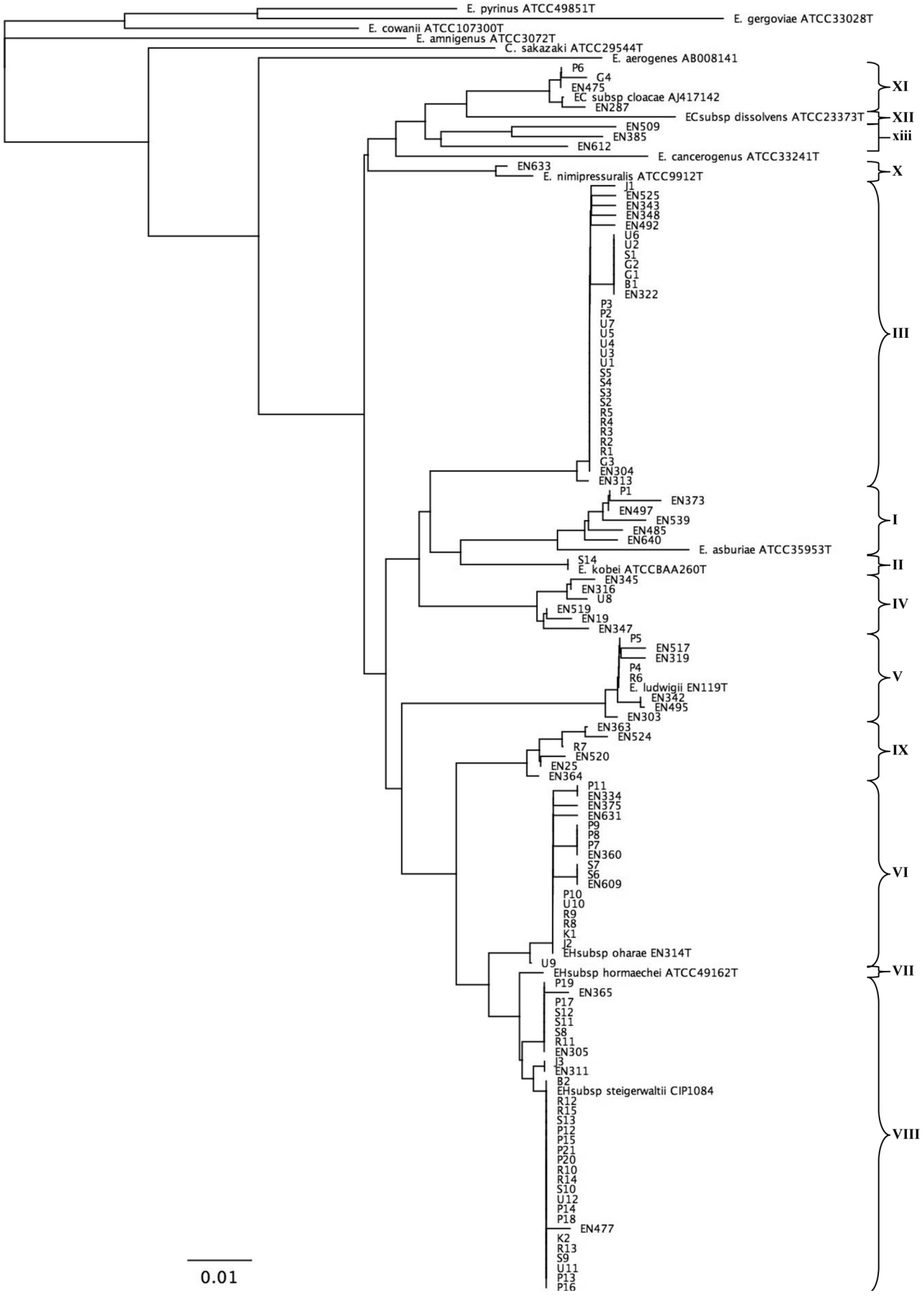
TABLE 2. Characteristics of control strains[a]

Characteristic	Value
No. of patients	52
M/F[b] ratio	1.4
Mean age at time of isolation ± SD (yr)	65 ± 18
Anatomical site of isolation (no. of isolates)	
Skin and soft tissue	14
Upper and lower respiratory tract	15
Urine	12
Joint or bone, in the absence of infected material	3
Intravenous catheter	2
Blood	2
Gastrointestinal tract	4

[a] One isolate from each patient was studied.
[b] M, male; F, female.

Predominance of *E. hormaechei* in hip prosthetic infections. Various anatomical sites were involved in orthopedic implant infections. Surprisingly, *E. hormaechei* (clusters VI and VIII) was the species involved in all cases ($n = 9$) of prosthetic hip infections, whereas strains isolated from other anatomic locations also belonged to other clusters (Fig. 2). The epidemiological association of *E. hormaechei* with hip implants was statistically significant compared to its association with other anatomical implant sites (9/9 cases at hip implant sites versus 6/12 cases at other anatomical implant sites; $P = 0.019$), but *E. hormaechei* infection of hip implants could not be linked to other demographic or clinical factors, such as age, a history of wound infection, or an association with other causative agents of infection. *E. hormaechei* subsp. *steigerwaltii* (cluster VII) was the taxon that was more frequently involved (six of nine cases) than *E. hormaechei* subsp. *oharae* (cluster VI; three of nine cases). The subspecies *E. hormaechei* subsp. *hormaechei* was not found in this series.

Molecular identification of routinely identified clinical *E. cloacae* isolates. In order to investigate the species distribution among the strains isolated from routine clinical samples, we performed sequence analysis of the *hsp60* gene from the control group, which consisted of 52 randomly selected clinical strains (Table 2). Similar to strains isolated from infected osteoarticular implants, *hsp60* sequencing showed that all strains


E. pyrinus ATCC49851T
E. gergoviae ATCC33028T
E. cowanii ATCC107300T
E. amnigenus ATCC3072T
C. sakazaki ATCC29544T
E. aerogenes AB008141
P6
G4
EN475
EC subsp cloacae AJ417142
EN287
ECsubsp dissolvens ATCC23373T
EN509
EN385
EN612
E. cancerogenus ATCC33241T
EN633
E. nimipressuralis ATCC9912T
J1
EN525
EN343
EN348
EN492
U6
U2
S1
G2
G1
B1
EN322
P3
P2
U7
U5
U4
U3
U1
S5
S4
S3
S2
R5
R4
R3
R2
R1
G3
EN304
EN313
P1
EN373
EN497
EN539
EN485
EN640
E. asburiae ATCC35953T
S14
E. kobei ATCCBAA260T
EN345
EN316
U8
EN519
EN19
EN347
P5
EN517
EN319
P4
R6
E. ludwigii EN119T
EN342
EN495
EN303
EN363
EN524
R7
EN520
EN25
EN364
P11
EN334
EN375
EN631
P9
P8
P7
EN360
S7
S6
EN609
P10
U10
R9
R8
K1
J2
EHsubsp oharae EN314T
U9
EHsubsp hormaechei ATCC49162T
P19
EN365
P17
S12
S11
S8
R11
EN305
J3
EN311
B2
EHsubsp steigerwaltii CIP1084
R12
R15
S13
P12
P15
P21
P20
R10
R14
S10
U12
P14
P18
EN477
K2
R13
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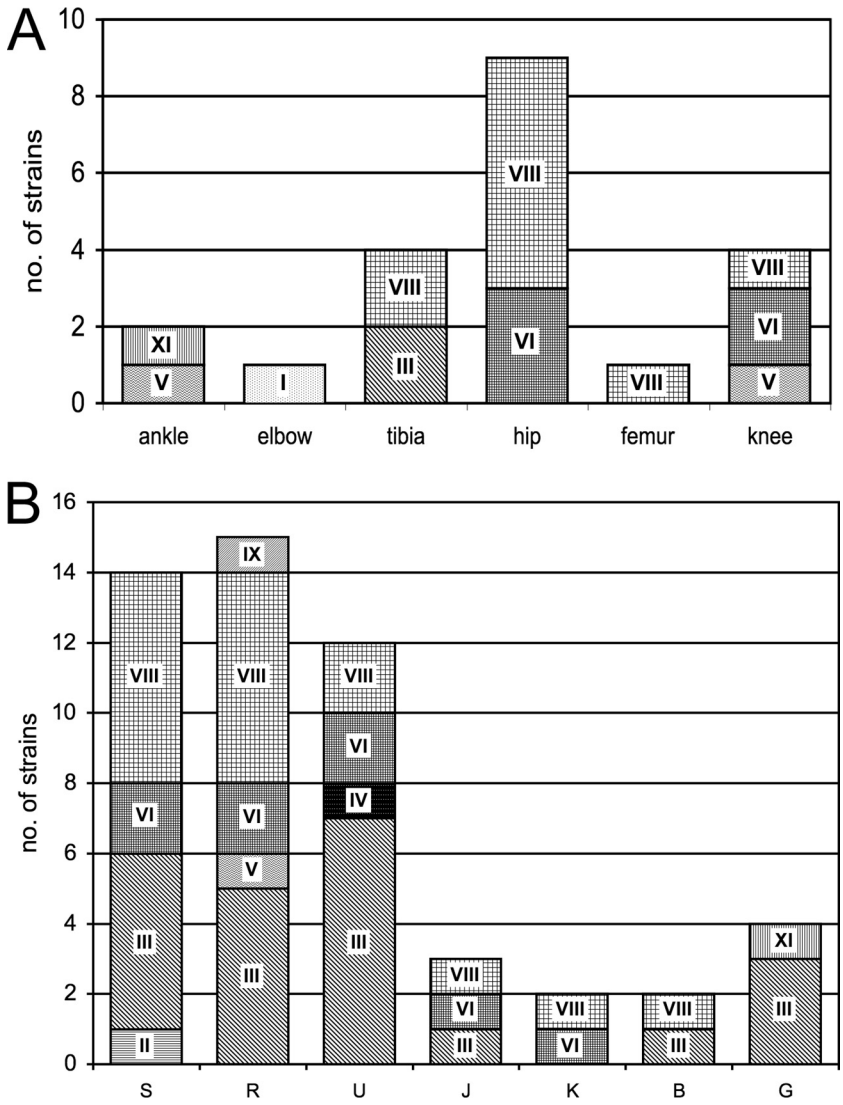

FIG. 2. Distribution of clinical strains within the genetic clusters of the *E. cloacae* complex. All strains could be assigned to one of the previously reported *hsp60* gene sequencing-based genetic clusters of the *E. cloacae* complex. (A) Strains isolated from implanted orthopedic material at different anatomical sites ($n = 21$). Hip-associated strains exclusively belonged to the *E. hormaechei* species (clusters VI and VIII). (B) Randomly selected clinical strains of diverse anatomical origins ($n = 52$). S, skin and soft tissue; R, upper and lower respiratory tract; U, urine; J, joint or bone, in the absence of infected material; K, intravenous catheter; B, blood; G, gastrointestinal tract. Irrespective of the site if isolation, cluster III accounted for 42% of the control isolates.

phenotypically identified as *E. cloacae* belonged to one of the molecular clusters of the *E. cloacae* complex (Fig. 1).

Without distinction by the site of isolation (Fig. 2), three clusters (clusters III, VI, and VIII) accounted for 90% of all strains. Similar to the implant-associated strains, genetic clusters belonging to the *E. hormaechei* species (clusters VI and VIII) were predominant (48% of control strains). Interestingly, cluster VII (*E. hormaechei* subsp. *hormaechei*) was ab-

FIG. 1. Neighbor-joining unrooted tree resulting from analysis of the *hsp60* gene sequences of 73 clinical strains and previously reported sequences. Isolation site of clinical strains: P, infected orthopedic implant; B, blood; J, joint or bone, in the absence of implanted material; G, gastrointestinal tract; R, respiratory tract; S, skin and soft tissue; U, urine; K, intravascular catheter. For the previously described strains, type strains are indicated (8, 10–12). Strains labeled EN were reported previously (8) and correspond to sequences with GenBank accession numbers AJ417125, AJ417127, AJ543819, AJ567887, AJ543876, AJ543894, AJ567893, AJ543787, AJ543803, AJ543806, AJ543829, AJ543864, AJ543882, AJ543804, AJ543789, AJ543781, AJ543784, AJ567846, AJ543776, AJ543808, AJ543877, AJ543807, AJ543866, AJ543775, AJ543878, AJ543881, AJ543820, AJ567878, AJ567885, AJ543857, AJ543831, AJ543821, AJ543816, AJ543798, AJ567847, AJ543777, AJ543861, AJ543768, AJ543855, AJ543870, AJ567881, and AJ543837. EH, *E. hormaechei*; EC, *E. cloacae*. The bar indicates the number of substitutions per site. Genetic clusters are numbered according to the description provided previously (8).

sent from our study, and *E. hormaechei* subsp. *steigerwaltii* (cluster VIII, $n = 18$) was predominant over *E. hormaechei* subsp. *oharae* (cluster VI, $n = 8$).

Cluster III accounted for 42% of control clinical isolates; thus, it occurred statistically more frequently within the control group of isolates than within the isolates from infected orthopedic implants (22/52 isolates versus 2/21 isolates; $P = 0.006$). These strains were recovered from various anatomical sites (respiratory, urinary and gastrointestinal tract, skin or soft tissue, and blood). The three bone and joint strains obtained in the absence of material (Fig. 2, series J [joint or bone]) were isolated from knee synovial fluid, knee soft tissue, and hallux valgus and belonged to clusters III, VI, and VIII, respectively; they thus represented each of the most frequently isolated clusters in the control group. The other clusters, cluster II (*E. kobei*, $n = 1$), cluster IV ($n = 1$), cluster V (*E. ludwigii*, $n = 1$), cluster IX ($n = 1$), and cluster XI (*E. cloacae* subsp. *cloacae*, $n = 1$), were poorly represented. Clusters I, VII, X, and XII and sequence crowd *xiii* were not found among the isolates in the control group. Taken together, these results show that cluster III, together with clusters VI and VIII, accounts for most of the strains routinely isolated from clinical specimens.

DISCUSSION

In this work, we investigated the distribution of strains involved in infections of osteoarticular implanted material within the genetic clusters of the *E. cloacae* complex. On the basis of *hsp60* analysis, we show that the cluster distribution for infected orthopedic implant-associated strains is different from that for randomly selected clinical strains of various anatomical origins. Our observation that all genetic clusters are not equally involved in pathogenesis highlights the need for more accurate routine bacterial identification tools and for a better understanding of the pathogenesis of the *E. cloacae* complex.

All strains evaluated in this study ($n = 73$) could be assigned to 1 of the 12 genetic clusters (clusters I to XII) of the *E. cloacae* complex. Only one strain was found to belong to cluster XI, the type strain of which is *E. cloacae* subsp. *cloacae*, which suggests that the widely used name *E. cloacae* is not representative of most clinical strains of the taxon. Preliminary identification as *E. cloacae* by conventional phenotypic identification methods (performed with the API 20E or the Vitek 2 system) was a prerequisite for the inclusion of strains in either the control or the study group. Although they might have led to possible underrepresentation of misidentified strains that authentically belong to the *E. cloacae* complex, the phenotypic identification methods performed with the API 20E and the Vitek 2 systems can be considered reliable tools for identification, as long as identification as "*E. cloacae*" is understood as "belonging to the *E. cloacae* complex."

Although patient populations vary from one hospital to another, the results of our studies of the distribution of the control strains within genetic clusters are concordant with those published previously, since we observed that clusters III, VIII, and VI account for most of the clinical isolates (8). Strains belonging to *hsp60* sequence analysis-based cluster III were shown to gather into the previously described MLSA-based cluster 1 as well as in CGH-based clade 2, with the latter being associated with strains that are the most frequently cultured in hospitals (21). Similarly, *hsp60* sequence analysis-based cluster VI (*E. hormaechei* subsp. *oharae*) and cluster VIII (*E. hormaechei* subsp. *steigerwaltii*) were shown to gather in MLSA-based cluster 2 but also to belong to the clinically relevant CGH-based clade 2. Thus, our data showing a predominance of cluster III, VI, and VIII isolates among control clinical strains of different anatomical origins are consistent with data presented in previous reports on the genetic diversity of the strains within the *E. cloacae* complex and support the congruence of CGH-based clade 2 with clinically relevant samples.

Some genetic clusters were absent from our study. Cluster VII (*E. hormaechei* subsp. *hormaechei*) harbors the original species type strain and was also poorly represented in other studies (8). Cluster X (*E. nimipressuralis*) is found in potable water reservoirs but, to our knowledge, has never been associated with human disease (13). Cluster XII (*E. cloacae* subsp. *dissolvens*), formerly part of the genus *Erwinia*, was reassigned to the *Enterobacter* genus and forms a subspecies of the *E. cloacae* species. It is associated with plants (maize, coffee), but no human infections have been reported (7, 9).

Although it was the largest cluster within the group of control strains (42%), cluster III was poorly represented within the group of orthopedic implant-associated strains (9%), and this difference was statistically significant ($P = 0.006$). Low numbers of cluster III isolates emphasize the large proportion of cluster VI (*E. hormaechei* subsp. *oharae*) and cluster VIII (*E. hormaechei* subsp. *steigerwaltii*) isolates, both of which belong to the *E. hormaechei* species. Hip joint prosthesis infections appeared to be specifically associated with *E. hormaechei*, since, in our series, all cases of infections of implants at this site were due to isolates of either cluster VIII ($n = 6$) or cluster VI ($n = 3$). The third subspecies of the taxon, *E. hormaechei* subsp. *hormaechei* (cluster VII), was not found in our analysis.

The low prevalence of cluster III isolates within the group of isolates from infected orthopedic implants compared to their prevalence within the group of isolates from clinical samples of other origins suggests a specific pathogenicity and reinforces the need for a robust and discriminatory tool for the accurate identification of isolates within the *E. cloacae* complex. In this regard, *hsp60* gene sequencing-based identification appeared to be both discriminatory and easily implementable, whereas other sequence-based molecular methods for the identification of *Enterobacter* were not as accurate. The absence of a consensus for the analysis of an *rpoB* DNA fragment (1 kb or 500 bp) has led to contradictory results, particularly for the genetic discrimination of isolates within *hsp60* gene sequencing-based clusters III, VI, and VIII (8, 21), as well as to the confusing use of the species name *E. hormaechei* for strains that do not belong to *hsp60* gene sequencing-based clusters VI to VIII (20). Similarly, sequence analysis of the gene encoding the DNA gyrase subunit B (*gyrB*) led to the hypothesis that most clinical isolates assigned to the *E. cloacae* complex by phenotypic semiautomated methods would belong to the *E. hormaechei* species (6). Analysis of the *hsp60* gene sequence was not included in this study, but the absence of cluster III as a specific group within the group of clinical strains evaluated in this study might suggest that *gyrB* sequencing is not as discriminatory as *hsp60* gene sequence analysis. Further investigation is needed to elucidate the latter point.

The epidemiological association between *E. hormaechei* and hip prosthetic implants allows new insights into previous observations to be made. First, the *E. hormaechei* species was reported in a case of prosthetic hip infection, although it was initially misidentified as *Escherichia coli* (25). This strain exhibited a phenotype of small-colony-variant formation, which was shown to be associated with regulation of the hemin uptake system (24). Although we did not systematically search for it, at least one of the strains involved in prosthetic infection displayed such a phenotype, with each step of subculture on solid medium leading to the emergence of fast- and slow-growing bacterial colonies. Second, although the study did not specifically refer to *E. hormaechei*, the ability of *Enterobacter* species to participate in biofilm formation on orthopedic implants was reported (1). Third, the ability of *E. hormaechei* to colonize implanted catheters as a biofilm and to be responsible for systemic infection was described (3, 5). The ability to grow as small-colony variants and the formation of biofilms are features frequently associated with the causative agents of orthopedic implant infections and contribute to the increased difficulty of diagnosis and treatment of such infections (18, 28). Taken together, the findings from the previous reports reinforce our observation showing the predominance of *E. hormaechei* as the cause of orthopedic implant infections. Further work is needed in order to identify the bacterial and host factors specifically involved in the bacterial colonization of the implanted material and in the pathophysiology of these infections.

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