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SPECIAL TOPIC: DISINFECTANTS AND MICROBIAL CONTROL

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Pseudomonas aeruginosa, *Candida albicans*, and device-related nosocomial infections: implications, trends, and potential approaches for control

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Abstract For many years, device-associated infections and particularly device-associated nosocomial infections have been of considerable concern. Recently, this concern was heightened as a result of increased antibiotic resistance among the common causal agents of nosocomial infections, the appearance of new strains which are intrinsically resistant to the antibiotics of choice, and the emerging understanding of the role biofilms may play in device-associated infections and the development of increased antibiotic resistance. *Pseudomonas aeruginosa* and *Candida albicans* are consistently identified as some of the more important agents of nosocomial infections. In light of the recent information regarding device-associated nosocomial infections, understanding the nature of *P. aeruginosa* and *C. albicans* infections is increasingly important. These two microorganisms demonstrate: (1) an ability to form biofilms on the majority of devices employed currently, (2) increased resistance/tolerance to antibiotics when associated with biofilms, (3) documented infections noted for virtually all indwelling devices, (4) opportunistic pathogenicity, and (5) persistence in the hospital environment. To these five demonstrated characteristics, two additional areas of interest are emerging: (a) the as yet unclear relationship of these two microorganisms to those species of highly resistant *Pseudomonas* spp and *Candida* spp that are of increasing concern with device-related infections, and (b) the recent research showing the dynamic interaction of *P. aeruginosa* and *C. albicans* in patients with cystic fibrosis. An understanding of these two opportunistic pathogens in the context of their ecosystems/biofilms also has significant potential for the development of novel and effective approaches for the control and treatment of device-associated infections.

Keywords Nosocomial infection · *Pseudomonas* · *Candida*

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Introduction

There is a general consensus that with increased antibiotic usage there is an increase in antibiotic-resistant microorganisms [25, 52]. The resultant current trend seen with nosocomial infections, which is consistent with this general consensus, is that the microorganisms most often associated with these infections are generally resistant to the common antibiotics of choice [25]. As selection of the appropriate antibiotic of first choice correlates with the highest degree of successful treatment, the selection of an effective antibiotic becomes increasingly more difficult when the pathogens most commonly encountered are not sensitive to the antibiotics of choice. At the global level, clinical experience with a wide variety of indwelling devices has shown that device-associated nosocomial infections are a significant reality; and it is estimated that at least 50% of nosocomial infections are device-related [39, 70]. For selected high-risk situations [e.g. central venous catheters (CVCs) in neutropenic patients], prophylaxis and/or antimicrobial-containing devices have been recommended and promoted to reduce the potential for infection [54].

The current theories regarding biofilm development [9, 41] and the generally regarded inherent resistance of attached/biofilm microorganisms to selected antimicrobials [18, 41] are further support for a significant role for the indwelling device and microbial attachment and biofilm formation in nosocomial infections.

Over the past 10 years, two opportunistic pathogens, *Pseudomonas aeruginosa* and *Candida albicans*, have been shown to be significantly involved in device-associated nosocomial infections for virtually all types of

indwelling devices. While microorganisms such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* spp are major concerns regarding nosocomial infections, the breadth of device-associated infections involving *P. aeruginosa* and *C. albicans* (which are ranked numbers 4 and 7, respectively, in infection control unit (ICU) nosocomial infections [53]) makes these two microorganisms obvious candidates and models for the study of device-associated nosocomial infections and the development of potential methods for the control of device-associated nosocomial infections.

The sections immediately following summarize *C. albicans* and *P. aeruginosa* device-associated nosocomial infections for three major device types: urinary catheters (catheter-associated urinary tract infections; CAUTIs), CVCs and blood stream infections (BSIs), and mechanical ventilation (ventilator-associated pneumonia; VAPs). This is followed by the current understanding of biofilm development as it relates to device-associated infection with respect to these microorganisms and the role of quorum sensing/cell signaling in this process. The final section covers current research and projected research , which aims to exploit the emerging understanding of biofilm development and device-associated infections.

P. aeruginosa/C. albicans: overview of device-associated infections

Table 1 presents a summary of the comprehensive National Nosocomial Infections Surveillance (NNIS) system report [54], which covers the most common isolates associated with nosocomial infections and their changes in antibiotic resistance. The table compares percent resistance data for isolates in 2003 with the composite mean percent resistance data from the previous 5 years. The data clearly shows that, except for two minor exceptions, antibiotic resistance among the most common isolates is increasing. Furthermore, this report shows that resistance to third-generation antibiotics and multiple drug resistance are also increasing. For the period of the NNIS 2003 report, data was also collected from the participating ICUs for infections related to CAUTIs, CVCs, and VAPs and these data are summarized in Table 2.

Table 3 provides representative data from selected studies to illustrate both the global and local nature of the problem.

P. aeruginosa/C. albicans: CVC-associated infections

In 2000 [51], it was estimated that *C. albicans* accounted for 5–10% (15,000–30,000 patients per year) of all nosocomial bloodstream infections in acute care hospitals, and it was attributed with a mortality of 25% (3,750–7,500 deaths per year). Subsequently, Hajeh et al. [28], in a similar but more recent review, place the numbers for *Candida* BSIs closer to 10%.

When only survivors are considered, the average overall stay for patients' *C. albicans* bloodstream infections is 24 days [51]. The increased costs associated with candidemia are in excess of U.S.$ 34,000 per patient and bear an annual cost of U.S.$ 0.5–1.0 billion (1997 dollars) [28, 51]

The colonization by and incidence of *Candida* infections and candidemia in neutropenic patients, and bone marrow transplants (BMTs) in particular, is quite high. In an analysis of 296 BMTs, colonization by *Candida* ssp was shown to approach 80% [75]. As a result of the high incidence of *Candida* infections associated with BMTs, fluconazole was administered prophylactically. With prophylactic administration of fluconazole, the incidence of *C. krusei* colonization increased significantly. It was further noted by Wingard et al. [75] that all of the *C. krusei* infections responded to amphotericin B. Based upon this new information, the protocol for BMTs was amended in that fluconazole was still

Table 1 Changes in antibiotic resistance in selected pathogen isolates from ICUs: comparison of resistance profiles for the period 1997–2001 vs the year 2002. Adapted from NNIS report, August 2003 [54]

Pathogen	Resistant antibiotic	Percent mean rate of resistance 1997–2001	Percent resistance (±SD) 2002	Percent change in resistance	No. of isolates (2002)
Enterococci	Vancomycin	23–27	27.5	+11	2,253
S. aureus	Methicillin	45–56	57.1	+13	4,303
Coagulase (–) staphylococci	Methicillin	86–90	89.1	+1	3,675
Escherichia coli	3rd generation cephalosporins	5–6	6.3	+14	1,439
Klebsiella pneumoniae	3rd generation cephalosporins	14–15	14.0	–2	990
P. aeruginosa	Imipenem	15–19	22.3	+32	1,500
P. aeruginosa	Quinolone	19–28	32.8	+37	2,064
P. aeruginosa	3rd generation cephalosporins	22–28	30.4	+22	2,383
Enterobacter spp	3rd generation cephalosporins	32–36	32.2	–5	1,485

Table 2 Range of pooled means for device-associated infections in ICUs for the period January 1995 through June 2003. The data was obtained from 11 types of ICUs; and the means are shown as: number of device-type infections/number of device-type days×1,000. Adapted from NNIS report, August 2003 [54]

Device	Range pooled means	Number of ICUs reporting
Urinary catheter	3.1–8.5	991
CVC	2.9–8.5	997
VAP	4.2–15.1	552

administered prophylactically to address potential infections by *C. albicans* or *C. tropicalis*, but if the patient became febrile, amphotericin B (or amphotericin B with flucytosine) was also administered. It was at this point that Wingard et al. [76] noted an increase in *C. glabrata* infections (*C. glabrata* comprised 75% of the 10% overall *Candida* infections noted in the BMTs receiving both fluconazole and amphotericin B).

Studies involving neutropenic patients clearly show a changing relationship regarding the *Candida* spp involved in CVC infections and the resistance profiles of these *Candida* spp. However, the role of indwelling devices in nosocomial infections is far less clear in neutropenic patients or "at risk patients" than with non-neutropenic patients. Other contributing factors, such as the geographic distribution of different *Candida* spp over the human body as it relates to the site of installation of an indwelling device, patient–patient contact, health care worker (HCW)–patient contact, mode of installation (surface, buried, tunneled), can also have an affect on which *Candida* sp. becomes the colonizing/infecting agent [15, 49]. To illustrate the effect of other factors on device-associated nosocomial infections, two recent examples are provided, as follows.

Contact with HCWs is estimated to account for >25% of these infections [51]. Rangel-Frausto et al. [66] reported the isolation of *Candida* spp in the hand-washings from HCWs in surgical ICUs at roughly 33%.

In a prospective study, conducted in Israel, of critically ill children who received a femoral CVC (>48 h in place), the rate of bacterial colonization for non-tunneled and tunneled procedures was 22.4% and 6.1%, respectively, with coagulase-negative staphylococci, *Pseudomonas* sp., and *Klebsiella* sp. the most common colonizers [49].

In a retrospective study of 404 selected cancer patients with candidemia [62], it was shown that removal of the CVC within 72 h of suspected BSI resulted in a significant positive response to anti-fungal therapy. In this study of candidemia and CVC-related BSI, all patients in the study were qualified as follows: all members of the study were first-time cancer patients, not on chemotherapy, non-neutropenic, not on steroid therapy, free of *Candida* infections for 30 days prior, and had the CVC installed for at least 24 h prior to symptoms of candidemia. In addition, the study included only those cases where candidemia was confirmed either by direct isolation and identification of *Candida* from the CVC or by differential blood culturing analysis. While earlier retrospective studies with non-nuetropenic patients failed to show the utility of catheter removal, these earlier studies did not address the question of the length of duration of the indwelling CVC prior to its removal. Interestingly, while Raad et al. [62] showed a statistically significant advantage of early removal of CVCs in non-neutropenic patients, no advantage was observed in removing CVCs from neutropenic BMT patients.

The findings of a selected number of global and regional surveys of candidemia are summarized in Table 3.

A 2-year, two-city (metropolitan San Francisco, Atlanta) prospective study of candidemia showed a similar incidence of candidemia (8:100,000 as determined against the entire metropolitan population) over the 2-year period in both cities [37]. Over the course of the study (1992–1993), there was a marked and significant decrease in the number of *C. albicans* BSIs. Of the 837 cases of candidemia, approximately 26% had cancer as an underlying cause. Interestingly, in comparing outpatient candidemia with nosocomial candidemia, there was virtually no difference in the distribution of candidemia based upon patient classification or underlying cause. *C. parapsilosis*, *C. glabrata*, and *C. tropicalis* were the most commonly isolated non-*albicans* agents of candidemia. The frequency distribution of the isolated *Candida* spp is shown in Table 3. Of all the candidemia isolates, those of *C. glabrata* and *C. krusei* showed ele-

Table 3 Global comparison of *Candida* isolates from cases of candidemia on a regional and national basis

	Raad [62], all patients		Raad [62], CVC-related confirmed		Kao [37]		Yang [78]		Rangel-Frausto [66], seven surgical ICUs	
	Number	%	Number	%	Number	%	Number	%	Number	%
C. albicans	128	32	44	43	183	46	15	29	20	48
C. parapsilosis	74	18	26	23	83	21	13	25	3	7
C. glabrata	71	18	11	10	59	15	8	16	10	24
C. krusei	53	13	5	5	10	2	1	2	–	–
C. tropicalis	45	11	11	10	49	12	12	23	8	19
Others	33	8	2	2	10	2	2	4	1	2

vated resistance to fluconazole (as compared with *C. albicans*).

The NEMIS survey [66] reported *Candida* spp as the fourth most common cause of BSIs (9.8 BSIs per 1,000 admissions), with *C. albicans* the most common cause (48%) and with the following general distribution for the non-*albicans* species: *C. glabrata* (24%), *C. tropicalis* (19%), and *C. parapsilosis* (7%; see Table 3). The colonization rate of surgical ICU patients was approximately 50%, while the MCWs in the surgical ICUs showed an overall rate of 33% positive *Candida* cultures from hand-washings (range 17–58% within the seven hospitals that participated in the study).

While the non-*albicans* species (*C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*) show a decreased sensitivity to fluconazole, these same species with the exception of *C. glabrata* show sensitivity to the newer generation azole antimycotics, such as ravuconazole [59]. In a study of invasive *C. glabrata* isolates (conducted in 2001–2002), the resistance/sensitivity profiles to the new azole antifungals demonstrated a significant geographic variability [60].

Yang et al. [78] reported on a survey of antibiotic resistance in *Candida* spp isolates from 20 hospitals in Taiwan. Consistent with other similar studies, this study showed that decreased fluconazole sensitivity was greatest among the non-*albicans* species. However, in the hospital survey in Taiwan, *C. tropicalis* isolates were the most commonly isolated fluconazole-insensitive *Candida* spp.

Parallel studies of bacteremia show a pattern with respect to neutropenic and non-neutropenic patients and with confirmed device association that is similar to that seen for candidemia. In a 5-year prospective study [22] of 242 BMT patients, 50% of the patients experienced a nosocomial infection. In neutropenic versus non-neutropenic days, the rate of bacteremia was 17.82/1,000 days and 5.51/1,000 days, respectively, and with catheter-related infections was 13.62/1,000 days versus 7.15/1,000 days, respectively. A 2-year retrospective study, in Taiwan, of bacteremia in neutropenic children showed 279 cases: *K. pneumoniae* (27.8%), *E. coli* (10.1%), *S. aureus* (10.1%) (half of which were multi-drug-resistant), and *P. aeruginosa* (7.6%) [7].

Edgeworth et al. [20] in a 25-year prospective study of bacteremia in an ICU reported two distinct stages. In the period 1971–1990, the number of bacteremias and the isolates recovered remained essentially the same, with *S. aureus*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae* predominating. In the second phase (1990–1995), the number of bacteremias doubled, primarily the result of increased isolation of enterococci, coagulase-negative staphylococci, and antibiotic-resistant *P. aeruginosa* and *C. albicans*. While the gentamicin resistance of the Gram-negative aerobes remained unchanged over the course of the study, the number of ceftazidime-resistant *Pseudomonas* spp doubled. Since 1986 (but reported only up to 1995), the causative agents in the majority of bacteremia cases (confirmed isolates) have been CVC-associated.

P. aeruginosa, *C. albicans*: mechanical ventilation associated pneumonias (VAPs)

Table 4 includes a listing of the most commonly isolated microorganisms associated with nosocomial pneumonias, in ICUs, in the United States, for the period 1992 through May 1999. In Table 2, the relative range is shown for VAPs (as number of device related infections per 1,000 device days) for the 552 ICUs reporting for the period 1995 through June 2003. Table 5 provides data from several regional studies on the most commonly isolated bacteria associated with VAPs in the respective study regions.

From Tables 4 and 5, which provide data from United States national and regional studies and from international studies, the list of microorganisms comprising the most common isolates associated with VAPs is in general consistent. However, when this analysis is conducted on a regional basis or is segregated according to patient profiling, there is considerable variability in the actual percentages and rankings of the most commonly identified isolates. This variability is particularly evident in the retrospective study of Babcock et al. [1] which noted significant variability in the VAP isolates from neonatal, pediatric, or adult ICUs (see Table 5).

Similar to the high rates of colonization seen with CVCs, colonization rates in mechanically ventilated patients can also be high. In a study involving critically ill, non-neutropenic, mechanically ventilated patients, El-Ebiary et al. [21] reported colonization rates by *C. albicans* and *P. aeruginosa* for all patients in the study group at 40% and 30%, respectively.

Similarly, in a 6-month prospective study of mechanically ventilated patients in two ICUs in Saint-Etienne, France, Berthelot et al. [2] reported the isolation of *P. aeruginosa* in 26 of 59 patients, a 44% rate of colonization, but only ten cases of confirmed *P. aeruginosa* VAP. In their 3-year prospective study, Valles et al. [73] reported *P. aeruginosa* colonization in 50% of the intubated patients. While it was shown that exogenous strains of *P. aeruginosa* accounted for 70% of the

Table 4 Most common isolates associated with nosocomial infections in ICUs in the United States for the period January 1992 through May 1999. Adapted from NNIS report, May 1999 [53]

Isolate	BSIs (%)	UTIs (%)	Pneumonias (%)
Enterobacter spp	4.9	5.1	11.2
E. coli	2.3	17.5	4.3
K. pneumoniae	3.4	6.2	7.2
P. aeruginosa	3.8	11.0	17.0
Haemophilus influenzae	n.r.	n.r.	4.3
S. aureus	12.6	1.6	18.1
CNS	37.3	2.7	n.r.
Enterococcus spp	13.5	13.8	1.7
C. albicans	5.0	15.8	4.7
All others	17.2	26.3	31.5
Number of samples	21,943	30,701	39,810

Table 5 Summary of relative ranking of the leading causes of VAP in selected studies

Isolate	Babcock [1], Washington ($n=753$)			Pawar [58], India (%; $n=25$)	Namiduru [50], Turkey (%; $n=140$)	Groot [27], Holland 16 ICUs (rank; $n=322$)	Chastre and Fagon [6], France (%; $n=1{,}689$)
	Adult (%)	Pediatric (%)	Neonatal (%)				
S. aureus	28.4	28.4	28.4	4	30		20.4
P. aeruginosa	25.2	33.3	17	22	33.9	1st	24.4
E. coli	2.3	9.5	2.3	10			3.4
K. pneumoniae	3.1	13	3.1	4			2.2
Achromobacter baumannii	10.2	1.7	1.7		26.1		7.9
Enterobacter				2	4.3		2.6

colonization isolates, 50% of the *P. aeruginosa* VAP cases were caused by endogenous strains.

Based upon chromosomal fragment pattern analysis by pulsed field gel electrophoresis (PFGE) of restricted chromosomal DNA obtained from endotracheal tube biofilms and lower respiratory tract secretions in 15 patients with recurrent VAP, Cai et al. [4] showed virtually indistinguishable patterns of *P. aeruginosa* for endotracheal tube biofilm isolates versus lower respiratory secretions in six of the 15 recurrent cases. Based upon their findings, Cai et al. [4] suggest that both environmental and endogenous sources of *P. aeruginosa* need to be considered when developing and assessing strategies for reducing VAP. This suggestion received previous support in the summary recommendations from the fifth NIAID workshop in medical mycology [51].

P. aeruginosa/C. albicans: CAUTIs

Tables 2 and 4 provide summary data regarding the rate of CAUTIs and the most common microorganisms associated with UTIs in the United States. The colonization rate of urinary catheters just associated to *Candida* spp is in excess of 30% [39]. The CAUTIs are the most common nosocomial infection in the United States, with over one million patients affected annually in ICUs and extended care facilities [47, 56, 77]. It is estimated [47] that the risk of CAUTI after 7 days increases 5% per day. While *P. aeruginosa* accounts for 11–12% of CAUTIs for both short- and long-term catheterization, *Candida* infections (which account for 9% of all CAUTIs for <7 days) increase to 25% of all CAUTIs for catheterization beyond 7 days.

Recommended procedures to minimize the potential for infection developed by the Center for Disease Control in 1980 [8] are still in effect in 2004. It is interesting to note that, in the recommendations from Clegg et al. [8], there is no endorsement for routine bacteriological monitoring. By contrast, the fifth NIAID workshop in medical mycology [51] strongly recommends continued surveillance within hospitals and medical centers on a national basis. Furthermore, the workshop encourages the development of more sensitive and reliable methods for the rapid identification of medically important fungi.

It is clear that the impact of CAUTIs in both numbers of patients infected annually and economic impact is extremely significant. This problem is further magnified by the large number of patients with asymptomatic candiuria (as defined by less than 10^5 cfu/ml of urine from a clean voided specimen) and the current debate surrounding the antibiotic treatment or the need for antibiotic treatment of asymptomatic candiuria [39]. Much of this debate centers on the recurrence of candiuria subsequent to antibiotic therapy and the potential for unresolved candiuria (and UTIs in general) as a pool for the development of and transfer of multiple antibiotic resistance.

Similar to the trend seen with CVCs and VAPs for increased infections involving inherently resistant non-*albicans* or non-*aeruginosa* species respectively, recent reports of intrinsically resistant/multiple resistant isolates are seen in CAUTIs. For example, over a 3-year period, Lombardi et al. [44] reported the isolation of carbapenem-resistant *P. putida* strains (predominantly from UTIs). Sequence analysis and mapping of multidrug-resistant VIM-1 metallo-β-lacatmase in *P. putida* infections over a 9-month period in a single hospital ICU and subsequently with other hospitals in Italy suggests horizontal gene transfer, in the group I pseudomonads and a common ancestry to In70 in *Achromobacter xylosoxidans*.

P. aeruginosa/C. albicans: primary attachment/biofilm development and device-associated infections

Device-associated infections are not a new phenomenon, with reports of these infections occurring over 50 years ago. Furthermore, it is generally recognized that biofilm-associated infections are recalcitrant to treatment [17, 41] and specifically that biofilm cells of *P. aeruginosa* and *C. albicans* can be significantly more resistant to/tolerant of antimicrobials than their respective planktonic cells [5, 19, 29, 30, 63, 65].

The recognition of the high rate of device-associated infections has resulted in the formation of a number of device-specific working groups tasked with reducing the incidence of infections associated with that particular device, and with each working group publishing guide-

lines for the prevention of these infections. For example, the working group which addresses BSIs recently recommended that, for adults receiving CVCs anticipated to remain in place for five or more days, the CVC employed should have an incorporated antibiotic or antiseptic [55]. In 1999, the NNIS report indicated that 50% of all nosocomial infections may be device-related [70]. This earlier estimate was reaffirmed in a recent review of device-associated *Candida* infection [39].

In a 30-month prospective study, conducted in The Netherlands, of ICU patients with an ICU-stay of ≥48 h, 27% of all ICU patients (42 infections per 1,000 ICU patient days) developed a nosocomial infection. In this particular study, the rate of infection per device type was VAP 43%, BSI 20%, and UTI 21%, with the major isolate for each device associated infection type being *P. aeruginosa, S. epidermidis*, and *E. coli*, respectively. Of the 2,795 patients under surveillance, 62% were mechanically ventilated, 64% had a CVC, and 89% had a urinary catheter [27].

In addressing the recalcitrant nature of biofilm-associated infections to treatment, Trautner and Darouiche [72] proposed a model for the development of biofilms associated with urinary catheters that is essentially the general model proposed for biofilm development by Costerton et al. [9], Denstedt et al. [14], Pratt and Kolter [61], Davies et al. [11], and Kolter and Losick [40]. This model recognizes four phases of biofilm development: (a) deposition on the catheter, (b) primary attachment, (c) cell division and recruitment, and (d) the mature biofilm as depicted by the ''coral model with communicating channels''. In their model, Trautner and Darouiche [72] suggest that devices impregnated with or coated with antimicrobials may be rendered ineffective during the first phase of biofilm development. With respect to some of the early devices impregnated with or coated with an antimicrobial, it has been suggested that early leaching/release of the agent from the device can also contribute to the loss in effectiveness [74].

The research of Jabra-Rizk et al. [34] with mature biofilm cells and Mateus et al. [48] with primary attached cells (but each with different strains of *C. albicans*) both showed a significant decrease in sensitivity (i.e. increased tolerance) to selected azole antimicrobials by the attached cells versus their respective planktonic cells. While not discounting the mechanisms suggested by Trautner and Darouiche [72] and Walder et al. [74] for the loss of effectiveness in devices incorporating antimicrobials, the decreased sensitivity to antibiotics/antimicrobials by cells attached to device surfaces may represent an important mechanism for the apparent antimicrobial resistance in device-associated infections and may contribute to the problem of drug resistance/tolerance.

Jabra-Rizk et al. [34] implicated the *CDR* genes, *CDR1* and *CDR2*, which both encode ATP-binding cassette transporters, for decreasing the fluconazole sensitivity of *C. albicans* in mature biofilms (24–48 h). Mateus et al. [48] showed that there is a third export transport protein, encoded by *CaMDR1*, which is also involved in decreased fluconazole sensitivity in primary attached cells (early biofilm development) of *C. albicans*. Whereas Jabra-Rizk et al. [34] had shown that decreased sensitivity to fluconazole required some time to develop, Mateus et al. [48] have shown that decreased fluconazole sensitivity develops quite rapidly in *C. albicans* CaI4 cells attached to medical grade silicone. Furthermore, using null mutants, it was shown that primary attached cells deficient in *CaMDR1*, *CDR1*, and *CDR2* exhibited the same level of fluconazole resistance as planktonic cells. Wild-type cells of *C. albicans* CaI4, when attached to medical grade silicone, showed two- and five-fold increased levels, respectively, in the expression of the promoters of *CaMDRI* and *CDR1*. Primary attached, isogenic mutants deficient in *CaMDR1* were ten-fold more sensitive to fluconazole than primary attached CaI4 cells, while double mutants deficient in both CaMDR1 and CDR1 were 100-fold more sensitive to fluconazole than primary attached CaI4 cells.

While multidrug-resistant (mdr) pumps have been shown to play a significant role in the decreased sensitivity of *C. albicans* cells in biofilms, a recent study [12] exploring the fitness and antibiotic resistance of *P. aeruginosa* cells in biofilms showed that the four characterized mdr efflux pumps in *P. aeruginosa* may not play a similar role in antibiotic resistance. Upregulation of *mexABoprM* or *mexCDoprJ* was not seen in the mature biofilm cells of *P. aeruginosa* generated in polycarbonate flow-cells at days 4, 6, and 8. In subsequent experiments involving mutants that were either deficient in or overexpressed only one of the mdr operons, no real difference was seen between antibiotic resistances for planktonic or biofilm-grown cells. However, in those constructs that overexpressed only one of the mdr operons, both planktonic and mature biofilm cells showed decreased antibiotic sensitivity to a selected number of but not all the antibiotics tested [12], suggesting that overexpression of a mdr pump in *P. aeruginosa* may contribute to decreased sensitivity to some antibiotics.

P. aeruginosa/C. albicans: new approaches for the control of biofilm development and device-associated infections

The role of microbial attachment and subsequent biofilm development is at the center of many research approaches aimed at addressing device-associated infections. The incorporation of anti-microbials or anti-infectives in a device or in coatings on devices continues to be an area of intense development.

The relative efficacy of CVCs incorporating antibiotics/anti-infectives in reducing infection was addressed by Walder et al. [74] in a retrospective analysis of 19 trials. The 19 trials entailed 22 device types: chlorhexidine/silver sulfadiazine-coated CVCs (12), silver-impregnated cuffs (5), multiple antibiotic-coated CVCs (5), and silver-coated CVCs (2). The analysis by Walder

et al. [74] indicated that there was a statistical reduction in BSIs for those CVCs coated with chlorhexidine/silver sulfadiazine and where the duration of catheterization was ≤ 6 days. In shorter-term trials involving antibiotic-coated CVCs, the reduction in BSIs was even higher. For longer-term duration, the results for BSI reduction are not apparent. For chlorhexidine/silver sulfadiazine- and antibiotic-coated devices, the effective active life of the agent is an important factor. Dissolution/loss of agent as a function of time is a real concern. The role of biofilm development with increasing duration of the catheter was not established from the retrospective analysis. However, the retrospective analysis did document colonization. As concluded in their multiple-trial review [74], the results of the studies analyzed gauged not so much the efficacy of antibiotic/anti-infective incorporation with CVC but that this technology is still quite new and still developing.

With respect to CAUTIs, Karchmer et al. [38] in a 12-month study of 27,878 patients showed a 32% reduction in the risk of infection when silver-coated cathers were employed. Previously, Maki et al. [46] had shown the same type of silver-coated catheter to be effective against *C. albicans*. In a recent review of CAUTIs, Maki and Tambyah [47] indicated that, while the role of biofilm on urinary catheters has not been fully established, the use of anti-infective impregnated and silver-hydrogel catheters significantly reduces the risk of CAUTI and represents the first significant advance in the prevention of CAUTI since closed drainage systems were fully implemented.

As stated previously, it is recognized that biofilm development is a process that occurs over a period of time. Based upon this premise, there is a growing interest in control methodologies that focus on the early stages of biofilm development and the changes that occur when microorganisms go from a planktonic state to being surface-associated/attached. Central to these approaches is the understanding of the mechanistic steps that occur during primary attachment and subsequent biofilm development.

In reducing device-associated infections, serious consideration needs to be given to the actual device surface (i.e. the materials/coatings). In addition to the incorporation of anti-microbials or anti-infectives, the receptivity (compliance) of the surface to microbial attachment and colonization must be understood. Considerable effort has been expended upon and reported on the development of surfaces/coatings that are less compliant with respect to microbial attachment. The large body of research documenting the different levels of attachment of specific strains, their mutants, and constructs, using a variety of materials under a variety of conditions is acknowledged but is beyond the scope of this review.

Examples of several recent approaches directed towards exploiting the process of colonization and biofilm development have taken several paths, and include: the disruption of quorum sensing, targeting mdr pumps, the use of iron scavengers/chelators impregnated into the device [36, 42, 45, 71], and the use of benign colonizers [10, 72].

Recently, with the broad interest in cell-sensing and the concurrent developments in molecular biology, the area has seen considerable activity. In the past several years, it has been shown that genes under the control of quorum-sensing regulation play an important, if not critical, role in microbial attachment, virulence, and colonization [11, 13, 57]. Given the importance of quorum sensing and cell–cell signaling, there has been considerable interest in attempting to affect microbial attachment and virulence through the control or manipulation of the microbial quorum-sensing system [16, 23, 24]. It has become clear, however, from parallel research involving bacteria–plant interactions, that stimulators, antagonists, analogues, mimics, and even extracellular hydrolytic enzymes are just a few of the mechanisms which can serve to mitigate or modulate quorum sensing- and cell–cell signaling-regulated activities.

For example, it has been shown that cross-signaling occurs between the two closely related bacterial species, *P. aeruginosa* and *Burkholderia cepacia*, when these two strains are grown together in a mixed culture under defined conditions [67]. Furthermore, it has also been shown that bacteria, such as *Salmonella enterica*, which do not synthesize acyl-homoserine lactone (AHL) signal compounds, do in fact possess AHL "receptors", which allow the cell to "listen" to other bacteria [3]. This was shown to occur in specific constructs in *S. enterica* var. *typhimurium* which showed sdiA activation when specific AHLs were added. This report was the first instance where a microorganism which does not produce AHLs exhibited gene expression in the presence of specific AHLs derived from other species. Recently, Hogan and Kolter [31] showed that *P. aeruginosa*, when grown in defined co-culture with the yeast *C. albicans*, can control the succession of *C. albicans* on selected surfaces by the preferential killing of *C. albicans* cells (while in the hyphal form but not in the yeast form). Previously, Hornby et al. [33] had shown that farnesol serves to mediate quorum sensing in *C. albicans*. Recently, Hogan et al. [32] have shown that farnesol (above 50 μm) significantly inhibits filamentation in *C. albicans* CAI-4 *HWP1-lacZ*, while Dennard and Pierce (unpublished data) have shown that 25 μm farnesol significantly impacts the expression proteome of *P. aeruginosa* PAO-1. The observations made by Hogan and Kolter [31] do not represent isolated findings but are supported by a large body of clinical and laboratory data from cystic fibrosis (CF) research and clinical practice. The incidence of *Pseudomonas* in CF patients is quite high (68–70%). Furthermore, recent studies have shown that *C. albicans* can be routinely isolated from the sputum of CF patients, yet the incidence of *Candida* infections in CF patients is low. However, recent clinical evidence shows that there is an increase in *C. albicans* infections in CF patients who are receiving antibiotic therapy to reduce

Pseudomonas infections. A number of researchers have shown that extracts of *P. aeruginosa* cultures contain anti-candidal properties.

In understanding the process of microbial attachment and biofilm development, an analysis of *Pseudomonas* and *Candida* infections in CF patients provides insight into a process that strongly suggests that some microorganisms can and do exhibit control over other microorganisms. The *Pseudomonas*/*Candida* infection model in CF patients affords the potential to understanding the mechanisms involved.

The use of quorum-sensing antagonists such as furanones has been proposed [68] to disrupt quorum sensing, resulting in a failure to develop a mature biofilm. However, recent information suggests that some furanone compounds may actually serve as nutrient sources for selected strains of *P. aeruginosa* [26] or may actually stimulate quorum sensing in some strains of *P. aeruginosa* and *B. cepacia* [69].

The interest in understanding the processes involved in microbial attachment and biofilms is not limited solely to developing new approaches to controlling device-associated infections but also concerns the development of improved diagnostic tools for the rapid and accurate identification of device-associated infections and in particular the identification of species showing resistance to the commonly prescribed antimicrobials.

Important to the development of improved methods for the control of device-associated infection is an understanding of not only the species involved but also the genetic diversity of these species. In an analysis of genetic diversity (as determined by PCR-RFLP) and biofilm-forming potential (method of Ramage et al. [64]) in *C. albicans* isolated from sewage, human oral cavity, and vaginal candiasis, Li et al. [43] showed considerable genetic diversity. The highest observed genetic diversity was with the vaginal isolates, and the least from the oral cavity isolates. Of the 115 isolates, there were 56 multilocus genotypes and of these 41 were unique. The number of shared genotypes between the three sources ranged from 34% to 51%. There was no correlation between source of isolate, clone group, or clonal lineage in the ability to form a biofilm on polystyrene.

In a retrospective study, 100 isolates, all identified as *P. aeruginosa* and all obtained from a single hospital in Ohio, were characterized according to their O-serotype [35]. Four representative cultures of the three serotypes which identified 0:3, 0:6, and 0:11 were grown on tryptic soy agar and then divided into equal aliquots. Isolated DNA from each of the aliquots was then subjected to PCR-based fingerprinting, using either arbitrarily primed PCR or enterobacterial repetitive intergenic consensus PCR. Half of the samples were then analyzed using either conventional slab gel electrophoresis or a microchip gel. Both methods showed agreement with conventional identification and serotyping. As the number of *P. aeruginosa* strains was small, additional testing was required in order to validate either method. Regardless, fingerprinting using either method is not likely to provide the resolution obtained by PCR-RFLP analysis.

Summary

At the national and international level, the numbers of and incidence of device-associated nosocomial infections is of serious concern. While there is a generally consistent list of microorganisms most commonly associated with a particular device, newer strains are being encountered. With respect to *C. albicans* and *P. aeruginosa*, these newer strains increasingly include non-*albicans* and non-*aeruginosa* species, respectively. With respect to all of the common isolates associated with device-related nosocomial infection, the incidences of antibiotic resistance (or decreased sensitivity) and multiple drug resistance are increasing.

The potential adverse role of microbial attachment and biofilm formation as it relates to device-associated infections and the development of antibiotic resistance is recognized but not fully understood. Considerable research is underway, and more is needed, in an effort to understand microbial attachment/biofilm formation on medical devices and to exploit this knowledge in developing new approaches to combat device-associated nosocomial infections. Research is also being conducted on understanding the *P. aeruginosa*/*C. albicans* interaction as it relates to infections in CF patients and to how some microorganisms control other microorganisms. Understanding the relationship between attached/biofilm cells and device surfaces/coatings is a key element in developing improved methodologies for the control of device-associated nosocomial infections.

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