

this M-3 clone also caused outbreaks of invasive disease in 1994 in Japan [3], and as we recently reported, in 1995 in Minnesota (designated PFGE-1) [4]. Kiska kindly provided us with the North Carolina M-3 clone, which we were able to compare with the Minnesota M-3 clone. Pulsed-field gel electrophoresis profiling confirmed that these organisms were indeed clonally related. Of additional interest, Musser et al. previously demonstrated that this same M-3 clone (designated ET2) caused frequent cases of invasive disease before 1993 [5]. All of these findings demonstrate the geographically widespread dissemination of this highly virulent streptococcal clone, an observation that may have important implications for future surveillance and preventive strategies.

According to their evaluation and a review of the literature, Kiska et al. also hypothesized that "cases of noninvasive disease may be serving as a reservoir for the dissemination of virulent strains to persons susceptible to invasive infection." Their proof for this assumption relates to several articles, including the present article, which describe the increased isolation of M-1 and M-3 GAS strains from cases of noninvasive (pharyngitis) or invasive streptococcal disease in the same geographic region at the same time. No information is presented about asymptomatic GAS pharyngeal carriers, which may represent a significant portion of the GAS reservoir in a community. To our knowledge, these findings were based on retrospective and not real-time analyses of GAS isolates obtained from patients with pharyngitis or invasive infection during defined outbreaks of invasive disease. Additional evidence in the literature for this "reservoir theory" was provided by Musser et al., who demonstrated that the predominant GAS clone carried by asymptomatic personnel also caused an outbreak of invasive disease in a closed military population in 1993 [6]. Also, our group recently showed that the 1995 civilian outbreak of invasive streptococcal disease in Minnesota was caused by an M-3 clone (the same M-3 clone reported by Kiska et al.) that at the same time accounted for 78% of GAS carried by asymptomatic school-aged children in the same community [4]. As well, we demonstrated in this report that during the outbreak, the M-3 GAS clone predominated as the cause for streptococcal pharyngitis in this community. In addition, on the basis of data presented in a recent article, Muotiala et al. [7] reached a similar conclusion in regard to M1 strains recovered in Finland. Both our study and the Musser et al. study were conducted in real time, that is, concurrent with the outbreak of invasive disease. Such real-time analyses permitted the selection of the most critical populations for GAS surveillance.

Considering all of the above, we agree with the conclusion of Kiska et al. that prospective surveillance of throat cultures for virulent clones of GAS, such as the M-3 clone they describe, may serve to alert medical care personnel about the potential for invasive GAS disease in a region or community.

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Antibiotic Resistance and O Antigen Expression in *Escherichia coli*

To the Editor—Recently, we published a phylogenetic analysis of putative enteropathogenic *Escherichia coli* recovered from Seattle children [1]. Five of the 5 *E. coli* recovered from patient 135 expressed the H2 flagellar antigen, belonged to the same electrophoretic type on multilocus enzyme electrophoresis analysis, and possessed *eae*, the gene encoding intimin. Four of these isolates expressed O antigen 25, and 1 expressed O antigen 128. The *E. coli* O25:H2 isolates produced ornithine decarboxylase and were resistant to ampicillin, amoxicillin-clavulanic acid, and cefazolin; the *E. coli* O128:H2 strain did not produce ornithine decarboxylase and was susceptible to these antibiotics. We hypothesized that a mobile genetic element, in particular a plasmid encoding antibiotic resistance, might be able to confer upon the *E. coli* O128 strain the ability to produce ornithine decarboxylase and to express the O25 antigen. Indeed, in *Salmonella enterica* serovar Borreze, a plasmid is responsible for serotype conversion [2].

To test this hypothesis, we mobilized a plasmid conferring ampicillin resistance from strain TB135B (*E. coli* O25:H2) into *E. coli* strain 395-1, a nalidixic acid-resistant derivative of strain AB1133 [3], via conjugative transfer. Subsequently, plasmid DNA from the ampicillin-resistant *E. coli* 395-1 was used to transform strain TB135A (*E. coli* O128:H2). The transformed, antibiotic-resistant *E. coli* O128:H2 was then serotyped and tested for the ability to produce ornithine decarboxylase [1].

The transformed *E. coli* O128:H2 continued to express the O128 antigen and did not coexpress the O25 antigen. Similarly, the transformed strain did not produce ornithine decarboxylase. These

data suggest that the expression of different O antigens in *E. coli* from patient TB135 is not the result of plasmid-mediated serotype conversion, the common electrophoretic type, H antigen, *eae* genotype, and patient origin of these organisms notwithstanding.

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Prevention of Aspergillosis in Bone Marrow Transplantation

To the Editor—In the June 1997 issue of the *Journal*, Wald and coworkers provided a comprehensive, although retrospective, analysis of aspergillosis in recipients of bone marrow transplantation [1]. One of the conclusions of this interesting article was that the use of laminar air flow (LAF) rooms is protective against early, but not late, aspergillosis, during the transplant course. I think that this conclusion should be substantially modified because it is not adequately supported by the data presented.

First, in this article, the allocation of patients to either LAF or standard rooms was not randomized. Patients with aplastic anemia and those receiving grafts from unrelated donors were put in LAF rooms, but the allocation of other patients was left to the discretion of the attending physician. Clearly, this decision could have been biased by many factors, including the physician's personal opinion about the usefulness of LAF rooms, the ability of the patient to accept this psychologically demanding situation, and the patient's clinical conditions. Certain patients refuse to be put in an LAF room or are too sick to be cared for in such an environment.

Second, no information was provided about the criteria used to classify patients as "LAF" or "non-LAF." How long should a patient stay in an LAF room to be considered an LAF room patient? For example, if a patient is taken out of an LAF room after only 3 days because of lack of compliance or deteriorating clinical conditions and then develops aspergillosis after another 15 days, is he considered an LAF or a non-LAF patient?

Third, probably due to the shortage of space, the authors did not describe the type of environment in which patients outside the LAF rooms were cared for. It would be important to know whether

or not these patients still were in a high efficiency particulate air (HEPA)-filtered environment. Indeed, in an LAF room, the patient not only breathes in air that is filtered through HEPA filters but also air that is forced in a laminar unidirectional pattern from one wall to the opposite one. In addition, to live in an LAF room means to live in a room that is equipped with sleeved gloves hanging from a plastic wall and to be kept in a "sterile" environment, which usually includes sterilization of every item entering the room, a sterile diet, skin disinfection, and so on. The reduction in the level of circulating spores seen in this environment, which is likely to be responsible for the reduction in the incidence of aspergillosis [2, 3], is unlikely to be affected either by the laminar flow or by the isolation and sterilization procedures, but rather by the air filtration through the HEPA filters [3]. One can use HEPA filters without laminar flow and other isolation procedures and still obtain a reduction in circulating spores (and consequently aspergillosis) [4].

In conclusion, even if the multivariable analysis showed that LAF rooms were associated with a reduced risk of early aspergillosis, it is unlikely that this benefit resulted from the use of the LAF room system as a whole, but rather by the air filtration through the HEPA filters. This point is extremely important because LAF rooms are uncomfortable for the patient and expensive for the health care system, as pointed out several years ago [5]. In my opinion, the use of LAF rooms in bone marrow transplant patients should not be encouraged without convincing data supporting their efficacy in the prevention of infection.

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Reply

To the Editor—We appreciate the thoughtful comments of Viscoli [1] regarding our manuscript describing risk factors for *Aspergillus* infections in a cohort of patients undergoing bone marrow transplantation [2]. It is correct that the allocation of patients to