

SARS-CoV. The prevalence of HMPV infection in SARS patients validates the interest in HMPV's possible role in SARS etiology.

From November 2001 to February 2002, 1 year before the first cases of SARS appeared, we tested the sputum of patients >64 years of age who had experienced exacerbation of chronic obstructive pulmonary disease, for HMPV. Investigations were conducted on 90 episodes in 89 elderly patients, 62 males and 27 females, in which we found no other microorganisms that could have been related to the exacerbation of chronic obstructive pulmonary disease. RNA was extracted from the sputum samples and amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) to detect HMPV as previously described (3). Results of bacterial culture and culture and PCR to detect respiratory syncytial virus and influenza virus types A and B were negative, whereas HMPV was found in the sputum of five (three men and two women) immunocompetent patients, 77–87 years of age. The prevalence of HMPV infection was 5.5%, similar to the percentage obtained by Chan et al., when HMPV RT-PCR was conducted on the respiratory samples. Fever (temperature >38°C) was not present in any of the five patients infected with HMPV. Two patients were admitted to a hospital. Both patients had bronchial infection and cough with bronchospasm and moderate respiratory insufficiency (oxygen saturation rate: 90.3% and 88%, respectively) for >1 week. Sputum samples from an additional 70 elderly patients with exacerbation of chronic obstructive pulmonary disease with positive detection for influenza virus (n = 50) or respiratory syncytial virus (n = 20) were tested for HMPV infection. None of the samples showed HMPV infection.

Sequence analysis of amplicons from the five samples positive for HMPV infection showed >95% simi-

larity with HMPV sequences found in other parts of the world (4,5). Additional studies should be conducted to confirm that HMPV exacerbates chronic obstructive pulmonary disease. However, by performing an RT-PCR directly on the sample instead of the more efficient RT-PCR after viral culture used by Chan et al., these findings suggest that HMPV is a frequently undetected agent in acute respiratory infection unrelated to SARS. The important questions are whether HMPV and SARS-CoV coinfection would facilitate more severe SARS, or whether HMPV infection would facilitate a more efficient transmission of SARS-CoV.

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Integrations in *Salmonella* Keurmassar, Senegal

To the Editor: Infections caused by *Salmonella* are the primary cause of foodborne diseases; multidrug resistance to *Salmonella enterica* subsp. *enterica* is increasing. The selective pressure created by the widespread use of antimicrobial agents in animals and humans as prophylactic and therapeutic agents may have contributed to the dissemination of resistant bacterial strains. In 2000, the new serovar Keurmassar (35:c:1,2) of *S. enterica*, was described in Senegal (1). Integrations are efficient gene-capture systems by site-specific recombination and are involved in antimicrobial-drug resistance in gram-negative bacteria (2). Three classes of integrations are well characterized and are involved in antimicrobial resistance. Integrations have been found in different nontyphoidal serovars of *S. enterica* and recently in serovar Typhi (3).

We evaluated the contribution of integrations to the antimicrobial drug resistance of eight isolates of *S. enterica* serovar Keurmassar sent to the Senegalese National *Salmonella* and *Shigella* Reference Laboratory at the Pasteur Institute in Dakar from March to May 2000. One strain was isolated from poultry flesh, and seven strains were isolated from human stool or blood samples. Susceptibility testing was performed by disk diffusion method on Mueller-Hinton agar according to the Comité de l'antibiogramme, Société Française de Microbiologie, recommendations. The eight strains expressed an extended-spectrum β -lactamase, which was previously identified as SHV-12 (1). The strains were also resistant to aminoglycosides (amikacin, gentamicin, netilmicin, spectinomycin, streptomycin, and

tobramycin), chloramphenicol, sulfamethoxazole, tetracycline, and trimethoprim. Genomic diversity was studied by pulsed-field gel electrophoresis (PFGE) and analysis of *Xba*I restriction fragments as described previously (3). The eight strains isolated from poultry and humans specimens showed identical PFGE patterns, which suggested that all strains of *S. enterica* serovar Keurmassar isolated until May 2000 in Senegal belonged to the same clone.

Strains were screened for the integrons by polymerase chain reaction by using three sets of primers specific for the *int11*, *int12*, and *int13* genes coding for the integrase as described previously (3). The *int11* gene was detected in all strains. Class 2 or 3 integrons were not detected. Cassette assortment in class 1 integrons was determined by using the primers 5'CS and 3'CS complementary to the 5' and 3' segments as described previously (3). With these primers, we obtained two amplification products of 1 kb and 1.7 kb for each strain, which suggested that all strains contained at least two class 1 integrons. Sequencing of these amplification products showed that the first product of 1 kb contained the *aadA2* cassette, which confers resistance to streptomycin and spectinomycin. The second amplicon of 1.7 kb carried a new arrangement of two cassettes: *aac(6')*-*Ilc*, which confers resistance to gentamicin, netilmicin, and tobramycin; and *ereA2*, which encodes resistance to erythromycin. Class 1 integrons were previously found in strains of *S. enterica* of different serovars: Agona, Albany, Brandenburg, Enteritidis, Goldcoast, Hadar, Infantis, Ohio, Panama, Poona, Saintpaul, Typhi, Typhimurium, Virchow, and Worthington (3–7). All these integrons, except that of serovar Infantis, contained a streptomycin-spectinomycin resistance determinant, *aadA2*

or mostly *aadA1*, alone or in combination with other gene cassettes. The cassette *aac(6')*-*Ilc* was previously described in a single class 1 integron in *Pseudomonas aeruginosa* (AF162771). The *ereA2* cassette was first described in *Providencia stuartii* (8) in a class 1 integron. This cassette was since described in class 1 integrons of clinical gram-negative isolates and recently in a class 2 integron in *Escherichia coli* (9). To determine whether the resistance determinants carried by the integrons were transferable, we performed a conjugation experiment from *S. enterica* serovar Keurmassar to an *E. coli* strain resistant to nalidixic acid. We first used a selective medium containing nalidixic acid 50 µg/mL plus 25 µg/mL of streptomycin, one of the two integrons carrying the *aadA2* cassette. All antimicrobial drug resistances were transferred at once from each strain to *E. coli*. The analysis of plasmid content prepared by alkaline lysis method from all transconjugants showed a single plasmid of >30 kb. The polymerase chain reaction analysis of the plasmid DNA confirmed the transfer of the two integrons, which suggested that the integrons were borne by a conjugative plasmid.

The multidrug resistance of these strains could be explained by the fact that antimicrobial agents are used extensively in the poultry industry in Senegal to reduce deaths and to increase productivity (1). Moreover, in Senegal, as in many countries in Africa, antimicrobial agents are sold over the counter, which leads to self-medication, thus increasing the selective pressure.

This is the first finding of integrons in the newly described serovar Keurmassar of *S. enterica*. One integron contained two cassettes, *aac(6')*-*Ilc* and *ereA2*. This was also the first finding of such an integron with a new arrangement of these two cassettes in a clonal strain of *S. enterica* serovar

Keurmassar that had recently emerged. Indeed, the *aac(6')*-*Ilc* cassette was described only once in *P. aeruginosa*. Moreover, aminoglycosides are not used extensively in Africa because they are very expensive. Therefore, determining how this cassette combination was selected is difficult. The strains studied were resistant to multiple antimicrobial agents, including broad-spectrum cephalosporins by production of the extended-spectrum β-lactamase SHV-12. The two class 1 integrons described here could account for the resistance to only a few drugs. The *bla*_{SHV-12} gene was not carried by an integron. Otherwise, ampicillin, trimethoprim, and tetracycline are the antimicrobial agents commonly used to treat diarrheal diseases in Africa. All of the strains studied were resistant to these antimicrobials agents. Trimethoprim resistance *dfr* genes are frequently found in integrons (2). However, in this study, we were not able to detect integrons containing *dfr* cassettes.

The presence of a conjugative plasmid and integrons in this serovar is of clinical importance. Indeed, the spread of the multiple antimicrobial agent resistance to other *Salmonella* serovars or gram-negative bacteria might easily occur by the transfer of such a plasmid. Moreover, integrons could allow the acquisition of new genes.

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Hepatitis B Infection, Eastern India

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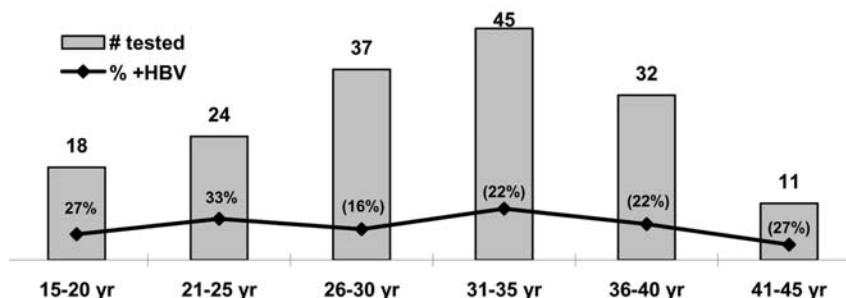


Figure. Distribution of sex workers by age and HBV infection (n = 167)

conducted a serologic study in July 2003 to determine the rate of hepatitis B virus (HBV) infection of brothel-based commercial sex workers. These study participants worked in the South-24 Parganas district of West Bengal, one of the eastern states of India. Routine immunization to prevent HBV infection is not a practice in India, and chronic HBV infection is endemic (1). The nature of their work makes commercial sex workers more vulnerable to HBV infection, which could accelerate the infection's spread into the general community, particularly in areas with low literacy rates and socioeconomic status.

The study participants were 167 commercial sex workers from three prominent brothels, which were located in small towns and along the national highway of the district. Blood samples from the participants were tested by using the HBsAg unlinked anonymous method. The results showed that 23.3% (unpub. data, National Institute of Cholera and Enteric Diseases) of the commercial sex workers were infected with HBV. The Figure shows the distribution of commercial sex workers and prevalence of HBV infection by age group.

To ascertain a possible route of transmission other than sexual activity, all study participants were questioned about their medical history, including jaundice, injury requiring blood transfusion, surgical events, and tattooing. The evidence indicated that HBV infection in the study participants was not acquired by any route

other than sexual activity. Since the carrier rate of chronic HBV in the general population is approximately 5% in eastern India (2), this increase in chronic HBV infection can be attributed to sexual transmission.

The Figure indicates that HBV infection is not correlated to age or duration of commercial sex work; the rate of infection is distributed almost equally in all age groups except the 26- to 30-year-old group in which it was slightly lower. Most of the HBV-infected commercial sex workers likely were infected early in their profession and remained infected throughout their lives, which led to higher rate of chronic infection as compared with the general population.

This higher rate of chronic HBV infection among commercial sex workers (23.3%) is of concern, particularly in a country with an estimated 4.58 million persons infected with HIV. In India, >80% of HIV infection is transmitted sexually (3). HIV infection can affect the natural course of HBV; sexual activity between HIV- and HBV-infected persons could prolong the infected status of those infected with HBV, as was shown in a previous study (4). This sexual activity could facilitate HBV transmission, particularly in areas that have few resources or where the rate of condom use is low or questionable.

After this study concluded, all study participants were notified of their infection status and advised to use condoms when engaging in sexual activity. The commercial sex work-