

## The Genus *Klebsiella*: An Ever-Expanding Panorama of Infections, Disease-Associated Syndromes, and Problems for Clinical Microbiologists

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### Abstract

One of the evolving concepts in clinical and diagnostic microbiology is the fact that many pathogenic genera of bacteria cause specific diseases or syndromes associated with certain subtypes, pathotypes, or clones of specific genomospecies. This has already been clearly established for *Escherichia coli* in regards to intestinal (IPEC) and extraintestinal (ExPEC) infections. Similar types of analyses are now being conducted on other pathogenic groups including the genus *Klebsiella*. In this article new information on the two most common *Klebsiella* species, *K. pneumoniae* and *K. oxytoca*, is reviewed including new disease manifestations or infectious syndromes, epidemiology, and virulence factors linked to these illnesses. The review also highlights challenges facing clinical microbiologists in screening for and detecting these pathotypes as they relate to clinical diagnoses.

**Keywords:** *K. pneumoniae*, *K. oxytoca*, Pyogenic liver abscess, Antibiotic-associated hemorrhagic colitis

### Introduction

The molecular revolution in clinical and diagnostic microbiology has been fueled by such innovative technologies as polymerase chain reaction (PCR), matrix-assisted laser desorption ionization-time of flight mass spectroscopy (MALDI-TOF) and 16S rRNA and full genome sequencing [1,2]. This revolution has spurred numerous advancements in the field of microbiology in the area of rapid diagnostics, description of new and novel pathogenic microbes and the phylogenetic characterization of pathogenic genera and species. This last category has led to the discovery of hypervirulent clones such as *Clostridium difficile* NAP1/O27 [3] and the subspecies characterizations of taxa into distinct pathotypes of clinical and medical importance. The best characterized of these species that has been subdivided into phenotypically and genotypically distinct pathotypes is the preeminent pathogen of the family *Enterobacteriaceae*, *Escherichia coli* [4]. In its most simple form *E. coli* has been divided into two major groups composed of Intestinal Pathogenic *E. coli* or IPEC and Extraintestinal Pathogenic *E. coli* or ExPEC [4]. The former group consists of such pathotypes as Shiga Toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), and enteroadherent *E. coli* (EAEC) while the latter group is comprised of uropathogenic *E. coli* (UPEC) and bloodstream/meninges-associated *E. coli* (MNEC, BMEC). Novel groups continue to be added to each category, in particular IPEC.

In addition to *E. coli*, species-associated pathogenic subtypes are beginning to become delineated at the phenotypic and/or molecular levels for several taxa that may have important ramifications for clinical microbiologists in the future regarding their identification. Two such groups are *Proteus mirabilis* [5] and *Yersinia enterocolitica* [6]. An even potentially more important observation is an expanding spectrum of disease presentations and illnesses associated with pathogenic *Klebsiella* species. This finding serves as the focus of this review.

### *Klebsiella*: Current Taxonomy

The genus *Klebsiella* has a checkered history in regards to species nomenclature and composition with many taxonomic issues remaining unsettled [7]. With the recent additions of *K. michiganensis* [8] and *K. quasipneumoniae* (with two subspecies) to the genus [9] the number of validly published species is now at 14 (<http://www.bacterio>).

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net/klebsiella.html). This does not include *K. alba* [10] which has no standing in nomenclature at present (<http://www.bacterio.net/-nonvalid.html>). Thus, the genus is currently comprised of 15 species in the broad sense (“sensu lato”) as listed in Table 1. However the reality is that two former species (*K. ozanae*, *K. rhinoscleromatis*) have been reclassified as subspecies of *K. pneumoniae*, three species have been transferred to the genus *Raoultella* (*K. ornithinolytica*, *K. planticola*, *K. terrigena*), two species are junior heterotypic synonyms of other previously proposed species, and another two (*K. mobilis*, *E. aerogenes*) have the same type strain and are homotypic synonyms of each other.

Recent population studies of over 300 strains of the taxon *K. pneumoniae* support the separation of this species into three separate nomenspecies, namely *K. pneumoniae*, *K. quasipneumoniae*, and *K. variicola* on a phylogenetic basis with *K. pneumoniae* remaining the predominant species associated with mammalian infections including humans [11]. Thus, the genus *Klebsiella* can at present be considered to be composed of five legitimate species including the three listed above and *K. michiganensis* and *K. oxytoca* with unresolved issues regarding the correct taxonomic position of *K. granulomatis* and *K. mobilis*. The vast majority of human *Klebsiella* infections are still attributed to *K. pneumoniae* and *K. oxytoca sensu stricto*.

## Clinical Infections

The genus *Klebsiella* and its two most note worthy species, *K. pneumoniae* and *K. oxytoca*, have long been recognized as important human pathogens [7]. These species have a long-standing association with serious hospital-associated infectious processes including blood stream infections (BSI), respiratory tract illnesses such as pneumonia and urinary tract infections [12,13]. The majority of these infectious processes in the past have been linked to long-term hospitalization and/or invasive medical procedures in general acute care hospitals [14].

The spectrum of infections associated with klebsiellae, however, is now changing rapidly as previously noted by Keynan and Rubinstein [15]. This “changing face” of *Klebsiella* involves the following clinical and laboratory-related issues (Table 2), (1) description of new or emerging disease syndromes associated

with one or more *Klebsiella* species, (2) a dramatic shift in the source of acquisition of some of these illnesses, that is, from the hospital- to the community-acquired environment, (3) identification specific pathogenic clones of virulence types triggering these infectious syndromes, and (4) the emergence of widespread resistance to first-line broad-spectrum antibiotics (e.g., carbapenems) previously used to treat many gram-negative infections. These changes will present major problems to the clinical microbiology laboratory in the future in regards to detection and identification.

## Klebsiella Pneumoniae

### Pyogenic Liver Abscesses (PLA)

**Disease:** PLA are pus-filled cavities containing infectious microorganisms that are located in one or more lobes of the liver typically as a secondary consequence of the translocation of pathogenic bacteria from the gastrointestinal tract into the circulatory or hepatic-portal systems. Common non-specific clinical presentations of PLA include fever, chills, and abdominal pain which may or may not be associated with the right upper quadrant of the liver (most common site for pyogenic abscesses). Previous reported mortality rates for treated cases of PLA range from 5% to 30% (Table 2).

In the past, PLA have been primarily associated with the recovery of *Escherichia coli*, streptococci, and anaerobic bacteria from infected material [16]. However starting in the mid-1980’s this bacteriologic picture began to change, particularly in Southeast (SE) Asia. During that time frame reports began to emerge from SE Asia from such locales as Taiwan and Singapore documenting *K. pneumoniae* as an important cause of community-acquired invasive PLA [17,18]. In one such study from Singapore 33% of all PLA cases were caused by *K. pneumoniae*, a frequency equal to that of *E. coli* [18]. Since then, the increasing role of *K. pneumoniae* in invasive PLA in SE Asia has been reported on numerous occasions. Today *K. pneumoniae* is the leading cause of PLA in Taiwan, South Korea, Hong Kong and Singapore [19]. Major risk factors associated with the development of *K. pneumoniae* PLA (KP-PLA) in these early SE Asian studies include Asian ancestry and diabetes mellitus [19].

Table 1. Current species composition in the genus *Klebsiella*

Species	Proposed Classification Change	Taxonomic Comment
<i>K. alba</i>		Species without standing in nomenclature
<i>K. granulomatis</i>	= <i>Calymmatobacterium granulomatis</i>	
<i>K. michiganensis</i>		
<i>K. mobilis</i>	= <i>Enterobacter aerogenes</i>	Homotypic synonym
<i>K. pneumoniae</i> ssp. <i>pneumoniae</i>		Type species
<i>K. pneumoniae</i> ssp. <i>ozanae</i>		
<i>K. pneumoniae</i> ssp. <i>rhinoscleromatis</i>		
<i>K. quasipneumoniae</i> ssp. <i>quasipneumoniae</i>		
<i>K. quasipneumoniae</i> ssp. <i>similipneumoniae</i>		
<i>K. ornithinolytica</i>	= <i>Raoultella ornithinolytica</i>	
<i>K. oxytoca</i>		
<i>K. planticola</i>	= <i>Raoultella planticola</i>	
<i>K. singaporensis</i>		Heterotypic synonym (junior) of <i>K. variicola</i>
<i>K. terrigena</i>	= <i>Raoultella terrigena</i>	
<i>K. trevisanii</i>		Heterotypic synonym (junior) of <i>K. (R.) planticola</i>
<i>K. variicola</i>		

**Table 2.** New Infectious Syndromes Associated with Major *Klebsiella* Species

Characteristic	<i>K. pneumoniae</i>	<i>K. oxytoca</i>
Syndrome	PLA	AAHC
Susceptible Population	Adults	Adults, children
Geographic Location	Primarily SE Asia	Worldwide
Risk Factors	Asian ancestry Diabetes mellitus	Penicillin therapy NSAID
Secondary Sequelae	Endophthalmitis Bacteremia Meningitis	Pseudomembranous colitis (?) Bacteremia (?)
Mortality (%)	<10	0
Bacterial phenotype/genotype	K1 (clonal)/K2	Cytotoxin-positive
Screening test(s)	String test	SCITB MACAA Spot indole

Abbreviations: AAHC, antibiotic-associated hemorrhagic colitis; NSAID, nonsteroidal anti-inflammatory drugs; MACAA, MacConkey agar with ampicillin and adonitol replacing lactose; PLA, pyogenic liver abscess; SCITB, Simmons citrate – inositol – tryptophan – bile salts agar

When this new syndrome was originally described it was thought at that time to be of only local or regional interest given the geographic locations from which published case reports originated from and the fact that one major risk factor was Asian ancestry. However beginning in the 1990's and continuing since that time an increasing number of community-acquired cases of KP-PLA have been reported from diverse locations around the globe including the United States, Ireland, Italy, Spain, Denmark, Germany, Argentina, and Oman among others [19-24]. The disease is now a global phenomenon. While a number of these cases involve persons of Asian ancestry or individuals that have traveled to SE Asia, many do not. One review cites approximately 50% of PLA cases in the US occurring in persons whose ethnic origin is Hispanic, black or white [19]. May of these cases are also appearing in relatively healthy middle-aged people with no history of serious medical conditions who subsequently abruptly develop symptoms suggestive of PLA [23].

The mortality rate for invasive KP-PLA has been recently estimated to vary from 4% to 8% in selected studies [19] although values as low as 0% to 2.5% have also been reported [20,21,25]. Besides the significant morbidity associated with this increasingly recognized invasive syndrome additional or suspected complications can result distinguishing them from primary monomicrobial cryptogenic or uncomplicated PLA.

In 10 to 25% KP-PLA cases patients subsequently develop metastatic infections as a consequence of primary infections [19,21,25]. Principle complications resulting from secondary spread include bacteremia, endophthalmitis, CNS disease, lung and prostatic abscesses, and fasciitis [19,26]. Predictive factors of metastatic infection included APACHE II scores, septic shock, diabetes mellitus, and platelet count <80,000/mm<sup>3</sup> while mortality is associated with APACHE II scores, septic shock, acute respiratory failure, and gas formation on imaging in separate studies [27,28]. Endophthalmitis is a particularly devastating secondary sequela as most outcomes result in blindness or severely impaired visual acuity [29]. Early recognition of the disease and a timely diagnosis are keys to minimizing the deleterious effects of such an infection.

Besides recognized infectious complications resulting from metastatic spread of KP-PLA the syndrome has additionally been associated with other morbidities. In an 11-year follow-up study from Taiwan colorectal carcinomas was found 2.68 times more

frequently in cases of KP-PLAs than they were for non-KP-PLA patients [30]. A similar association in another investigation found *K. pneumoniae* cryptogenic PLA and diabetes mellitus present in 7 of 8 patients with colon cancer or laterally-spreading tumors [31]. These findings suggest that the appearance of KP-PLA may be linked to colonic mucosal defects resulting from tumor formation. As such, these PLAs may be a potential predictor of colonic carcinomas requiring colonoscopy to detect occult malignant lesions. KP-PLA has also been found to have a higher risk of subsequently developing other infectious complications 90 days to one year post-diagnosis [32,33]. These illnesses include a variety of respiratory issues such as pleural empyema, pulmonary abscess, and pneumonia.

## Pathogenicity

A microbiologic and molecular analysis of many *K. pneumoniae* strains recovered from cases of PLA and other invasive diseases including bacteremia indicate that the causative strains of these infections are not randomly selected. Rather, strains associated with KP-PLA and related invasive sequelae have been called "hyper-" or "hepato-" virulent *K. pneumoniae* or hvKP [34-36]. hvKP strains possess a number of virulence characteristics or traits that are either unique to these strains or possessed at elevated frequencies when compared to standard or classic isolates of *K. pneumoniae*. These virulence characteristics include capsule formation, iron scavenging systems, biofilm formation, allantoin metabolism, and serum resistance [34,36]. One easily recognizable trait associated with these hvKP is the hypermucoviscous phenotype present as mucoid colonies on agar plates that may be the result of hyperproduction of capsular material. Of the 78 capsular types of *K. pneumoniae* the vast majority of hvKP strains belong to capsular type K1 and to a lesser extent K2.

A phylogenetic analysis of K1 and K2 PLA-associated strains recovered from different regions of the globe indicates that for the former capsular type isolates recovered from three continents suggests that these isolates are genetically similar with most belonging to Sequence Type (ST) 23 [37]. A recent study of a large collection of hvKP K1 strains and two hvKP K2 isolates by whole-genome sequencing indicates that the K1 strains belong to a monophyletic clade composed of a unique clonal lineage, Clonal Complex 23 (CC23) [38]. Minimal geographic grouping suggests that CC23 has spread globally through multiple international

transmission events [38]. In contrast to K1 CC23, K2 hvKP isolates are more genetically diverse. Regardless of the lineage, hvKP K1 and K2 strains all harbor a large 224-kb plasmid that contains three genes not found in classic non-PLA-associated *K. pneumoniae* strains. These plasmid genes include *rmpA* (regulator of the mucoid phenotype) and two siderophores, aerobactin and salmochelin [38]. The siderophore aerobactin has recently been shown to support growth and survival of hvKP *ex vivo* and *in vivo* [39].

### Laboratory diagnosis

Epidemiologic investigations show that gastrointestinal isolates of *K. pneumoniae* match liver isolates phenotypically (serotype) and genotypically as well [40] supporting previous hypotheses that most PLAs and systemic infections seed from the gut initially. This suggests that clinical microbiology laboratories should possibly screen high-risk patients (e.g., diabetes mellitus) for carriage of hvKP since at minimum 2% - 4% carry this genotype as normal flora [36]. Also KP-PLA should be typed to see if they belong to the hvKP group that may subsequently disseminate (bacteremia, endophthalmitis, meningitis) or increase long-term risk for developing other illnesses such as pneumonia.

Standard microbiological identification schemes such as automated systems, 16S rRNA gene sequencing, and MALDI-TOF will not identify hvKP. The best current approach is to screen isolated colonies on non-differential media (e.g., blood agar) for the hypermucoviscosity phenotype [41,42]. This test, classically known as the "string test", determines whether or not a viscous string of material >5 mm can be stretched out from a colony using an inoculating loop. This is very similar to the same test used for years to differentiate vibrios from aeromonads and plesiomonads. While far from ideal almost all hvKP produce hypermucoviscous colonies and the string test has been estimated in one study to have a sensitivity of just under 91% [42]. Once string test-positive strains are identified they should be typed for possession of K1 or K2 capsular antigens by standard serology (reference laboratories), PCR of the *wzy* and *wzx* loci [43], or DNA sequencing [44]. Unfortunately, in 2005 excellent *K. pneumoniae* capsular antisera (K1- K6) previously available from Denka Seiken (Japan) was discontinued.

### Lemierre's Syndrome

Lemierre's syndrome (LS) is an uncommon post-infectious complication of pharyngitis characterized by fever, chills, rigors, dysphagia, cervical neck discomfort, ipsilateral internal jugular vein thrombosis, and metastatic pulmonary abscesses [45]. In the past >90% of LS cases have been caused by *Fusobacterium necrophorum*, *F. nucleatum*, or other fusobacteria and LS has only rarely been associated with other microbes such as streptococci, anaerobic bacteria, or *Proteus* species [45]. Up until 2006, only two cases of LS caused by *K. pneumoniae* had been described. Since 2012, eight more cases of LS caused by *K. pneumoniae* have been published where it was the sole etiologic agent of the disease (vast majority) or a co-pathogen found in mixed culture [46,47].

Chuncharunee and Khawcharoenporn [47] have reviewed a majority of cases of *K. pneumoniae* induced LS. A cardinal observation in these persons is that 8 of 10 published cases have been reported from SE Asia (Malaysia, Taiwan, Singapore, Thailand) in middle-aged persons. In addition, of these LS patients

7 of 10 had diabetes mellitus as an underlying comorbidity, several of which were only recently diagnosed. These two risk factors bear striking similarities to those found associated with KP-PLA. It may well be that the increased incidence of KP-PLA in SE Asia may predispose persons to LS by some unknown mechanism. As KP-PLA expands internationally other parts of the world may also find *K. pneumoniae* as a potential cause of LS.

## Klebsiella Oxytoca

### Antibiotic-associated hemorrhagic colitis

Beginning in the late 1970's and thereafter sporadic reports began to surface in the literature describing the isolation of *K. oxytoca* from *Clostridium difficile*-negative persons with right-sided (segmental) hemorrhagic colitis subsequent to receiving antibiotic chemotherapy, typically penicillin derivatives [48,49]. These reports regarding antibiotic-associated hemorrhagic colitis (AAHC) increased in frequency over the next several decades (Table 2). In 2006, Högenauer and colleagues [50] proved that certain toxigenic strains of *K. oxytoca* were a cause of AAHC by fulfilling Koch's postulates using Sprague-Dawley rats.

The syndrome is characterized by an abrupt onset of bloody diarrhea with severe abdominal pain in *C. difficile*-negative individuals after receiving a short course of antimicrobial therapy [50,51]. Common drugs associated with the induction of AAHC include penicillin analogues such as amoxicillin and cephalosporins [51,52], although other class compounds such as quinolones and clarithromycin have been occasionally implicated [50,53]. Other prominent features of the syndrome include elevated leucocyte counts and C-reactive protein levels [50]. Risk factors associated with AAHC include recent antimicrobial therapy, concurrently receiving nonsteroidal anti-inflammatory drugs, and the presence of cytotoxigenic *K. oxytoca* in fecal specimens [50,51]. Recent studies in various patient populations indicate the frequency of *K. oxytoca* in the gastrointestinal tract to vary from 2.9% - 9.8% [54,55]. However the prevalence of cytotoxigenic *K. oxytoca* in these same populations is very low, ranging from 0.06% - 0.5% [54,55].

Unlike KP-PLA, at present there are no known secondary complications of AAHC. However, Sweetser and others [56] have described a case of pseudomembranous colitis secondary to *K. oxytoca* infection. A brief report from the University of Maryland has also suggested a possible association between *K. oxytoca* colitis and bacteremia [57]. Both of these are preliminary observations. The observed mortality rate associated with AAHC is 0% as individual cases respond immediately to cessation of chemotherapy.

### Pathogenicity

In the late 1980's the Japanese identified a unique cytotoxin from certain strains of *K. oxytoca* that produced cytotoxic effects on HEp-2 cells [58,59]. This cytotoxin had a proposed molecular mass of 217 Da [59]. Strains associated with AAHC are all cytotoxin-positive [50]. Recently the cytotoxin produced by *K. oxytoca* has been identified as the nonribosomal peptide tilivalline which causes apoptosis in cultured human cells *in vitro* such as HEp-2 [60]. The authors demonstrated that this enterotoxin also produces colitis in a mouse model. Thus *K. oxytoca* appears to produce AAHC in a similar fashion to that of *C. difficile* by overgrowth in the bowel subsequent to antimicrobial



therapy, thereby allowing for the production and release of a novel and unique enterotoxin causing colitis.

## Laboratory diagnosis

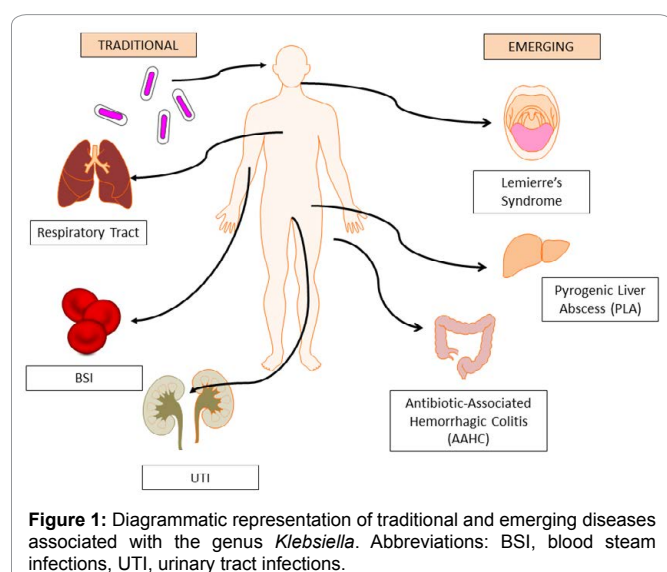
As in the case of KP-PLA, straightforward identification of *K. oxytoca* alone is insufficient in establishing a laboratory diagnosis of AAHC. *K. oxytoca* must not only be isolated from feces and separated from other enteric flora including *Klebsiella* species but must be demonstrated to produce cytotoxin in vitro. Fortunately the condition AAHC results in the overgrowth of *K. oxytoca* in the colon to numbers approximating  $10^7$  cfu/gm [60]. This single fact aids and helps facilitate the recovery of this pathogen from other microbial species. At least two selective media have been designed to help in this process. One has been called SCITB agar which stands for Simmons citrate agar supplemented with inositol, tryptophan, and bile salts [55]. Resulting inositol-positive colonies [yellow] are screened with spot indole reagent for positivity, indicating the possible presence of *K. oxytoca* (indole-positive species). Indole-positive colonies can then be definitively identified by conventional or automated testing [55]. The specificity of this method appears to be high [55]. A second method utilizes MacConkey agar containing ampicillin (30 mg/L) and substituting adonitol 91% wt/vol for lactose [54]. Colonies that appeared pink or red (adonitol fermentation) were subcultured to blood agar from which the spot indole test was performed [54]. Indole-positive were definitively identified as *K. oxytoca* by similar procedures to those listed above.

## Gastroenteritis

There is some very preliminary data suggesting that cytotoxin-positive *K. oxytoca* can produce a mild uncomplicated antibiotic-associated gastroenteritis distinct from AAHC [55]. Other earlier studies, however, have not found a similar association [61]. It is further unclear whether cytotoxin-negative strains play any role at all in bacterial enteritis.

## Conclusions

The transformation now occurring in the genus *Klebsiella* in regards to disease syndromes and presentations is depicted diagrammatically in Figure 1. While the genus *Klebsiella* has



traditionally been associated with a variety of healthcare-associated illnesses including bacteremia, pneumonia, and urinary tract infections (left side) [4], new syndromes are now being observed (right side). These new syndromes have a number of features in common which include (1) are more often community-acquired in many cases (PLA) than healthcare-associated, (2) are linked to defined virulence characteristics (capsule, enterotoxin) of select clonal or pathotypes of major species, and (3) require clinical microbiologists to perform screening and/or identification tests in addition to straightforward species identification to provide a definitive laboratory diagnosis for the physician.

In the clinical laboratory emerging molecular methods are geared for the rapid and specific identification of bacterial genera and species by means of PCR technology, 16S rRNA gene sequencing, MALDI-TOF, and soon next-generation full genome sequencing. However in many instances species identification is not sufficient alone as pathogenic groups or clones with linked virulence factors are the causal agents of specific diseases such as reported here for PLA and AAHC (Table 2). While culture-independent diagnostic testing may be able to identify certain prevalent pathotypes, such as Shiga toxin-producing *E. coli* (STEC), this method is unlikely to capture all specific groups associated with infectious maladies such as enteritis or bloodborne disease [62]. A further complication to this equation is the fact that, as an example, most strains of KP-PLA are currently susceptible in vitro to common antimicrobial agents yet recent data suggests that expression of extended-spectrum beta-lactamases (ESBL) and class-specific resistance to many compounds is beginning to be seen [63]. Such trends, if continuing could significantly impact treatment regimens and mortality rates associated with these entities.

Besides the use of evolving molecular methods clinical microbiologists will need to continue to rely on isolation techniques, screening reactions, and antimicrobial susceptibility testing to detect and identify the possible presence of new emerging pathogenic groups such as those causing PLA and AAHC. The infections presented above for the genus *Klebsiella* provide a good illustration of this fact. As molecular technology continues to identify more disease syndromes associated with specific pathotypes clinical microbiologists will be challenged to keep up with this progress by the use of multiple innovative strategies.

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