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Risk factors and new markers of pulmonary fungal infection

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Chapter 3

OUTBREAKS AND CLUSTERING OF *PNEUMOCYSTIS* PNEUMONIA IN KIDNEY TRANSPLANT RECIPIENTS: A SYSTEMATIC REVIEW

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Submitted

Abstract

From 1980 onwards, an increasing number of outbreaks of *Pneumocystis pneumonia* (PCP) among kidney transplant recipients have been reported. The cause of these outbreaks is unclear and different explanations have been provided. We performed a systematic review to provide a comprehensive overview of the epidemiologic characteristics as well as the involved clinical risk factors. A total of 15 peer reviewed English language articles published from 1980 onward were included. Outbreak settings were all marked by absence of chemoprophylaxis, frequent inter-patient contacts and lack of isolation measures taken during hospitalization of PCP cases. PCP associated mortality rates significantly decreased from a weighted mean of 38% before 1990 to 19% and 13% in the following two decades. Clinical risk factors for PCP in outbreak settings were largely similar to non-outbreak settings. Genotyping by multilocus sequence typing (MLST) or comparison of the internal transcribed spacer (ITS) regions 1 and 2 showed that the outbreaks are most frequently caused by a predominant or a single *Pneumocystis* strain. Pooled epidemiological data and genotyping results strongly attest to the emerging insight that acquisition of *Pneumocystis* occurs through interhuman transmission. No seasonal trend was noted. The results emphasize the need for chemoprophylaxis in kidney transplant recipients despite a low baseline incidence of PCP in this population and support the current CDC recommendation with regard to isolation of patients with PCP during hospitalization.

Introduction

Pneumocystis pneumonia (PCP), caused by *Pneumocystis jirovecii*, has been recognized as an important potential cause of morbidity and mortality in patients susceptible due to immunosuppressive medication needed after kidney or other (solid organ) transplantation [1]. Interestingly, and apart from the expected observation of solitary cases, an increasing number of sudden outbreaks or clusters* of PCP among kidney transplant recipients was reported from several continents. The pathogenesis of these outbreaks has not been clarified and different explanations e.g. changes in the standard immunosuppressive regimen, an environmental source or patient-to-patient transmission have been proposed [2-4]. Recent outbreaks occurred in the absence of chemoprophylaxis, while in general the prescription of Trimethoprim-Sulfamethoxazole (TMP-SMX) to prevent PCP for at least a duration of 3-6 months after kidney transplantation now is a widely accepted practice and incorporated in several kidney transplantation guidelines [5, 6].

During the first observations of clusters of PCP in kidney transplant units in the 1980s, where transplant recipients were hospitalized together with AIDS patients, the possibility of patient-to-patient transmission and a relation with the developing HIV epidemic in the northern hemisphere in general was proposed [7]. Outbreaks among kidney transplant recipients and other immunocompromised hosts were thereafter repeatedly observed but analysis was restricted to assessment of the epidemiological data and investigation of potential clinical risk factors [8-11]. The application of molecular genotyping methods from 1990 onwards pointed to the likelihood of either a common environmental source or interhuman transmission of PCP during an outbreak [12-16]. Until now an environmental source has not been identified and evidence from several studies demonstrated colonization of the human respiratory tract in asymptomatic healthy and immunocompromised individuals in up to 50% of the studied cases [17-20].

Combining these and other recent findings with the historical data of PCP outbreaks involving kidney transplant recipients, may lead to improved understanding of the factors contributing to *Pneumocystis* transmission and development of outbreaks of PCP among kidney transplant recipients (26). Main objective of this systematic review is to provide a comprehensive overview of the involved clinical risk factors as well as epidemiological characteristics of PCP outbreaks among kidney transplant recipients. Based on the results, we update current opinions with regard to the involved risk factors and mechanism(s) of transmission. This should lead to improved implementation – and maybe new - strategies for prevention of PCP in solid organ transplant recipients.

* When the term 'outbreak' is used, it is considered to represent both the terms 'cluster' and 'outbreak' throughout the manuscript.

Methods

A systematic literature review was performed to identify all English language papers describing PCP outbreaks in renal transplant recipients from 1980 onwards. An outbreak was defined as a reported unexpected sudden rise in incidence of PCP comprising at least 5 kidney transplant recipients. A comprehensive literature search using the PubMed and Medline databases was performed. The following exact search commands were used in both databases: (I) 'transplantation AND *Pneumocystis* AND outbreak', (II) 'transplantation AND *Pneumocystis* AND cluster', (III) 'kidney transplantation AND *Pneumocystis*'. Results were limited to English language articles, adult human subjects and time period from 1980 onward. The search procedure was repeated several times until the 1st of August 2010. A total of 145 peer reviewed articles were identified. All available abstracts were reviewed by the first author. Of the 32 articles of potential interest 14 fulfilled the inclusion definition. In case of doubt the last author was consulted. The PubMed option 'related articles', reference lists of included articles and consultation of expert sources were used to find additional articles missed by the initial search. One additional article was included by this secondary search (figure 1).

The following data was obtained from all 15 included articles: the total number of patients, year of publication, year of index case diagnosis, geographical region, PCP incidence prior to the outbreak, overall mortality, outcome of nested risk factor analysis, outcome of genotyping analysis performed on *Pneumocystis* organisms (if available) and environmental investigations. In table 1 (page 75) the major characteristics of the included outbreaks are described. A meta-analysis was not attempted due to the heterogeneity of transmission analyses. In the sections below the results and observations are discussed in the context of the current knowledge about *P. jirovecii*.

Figure 1. Summary of systematic literature search results.

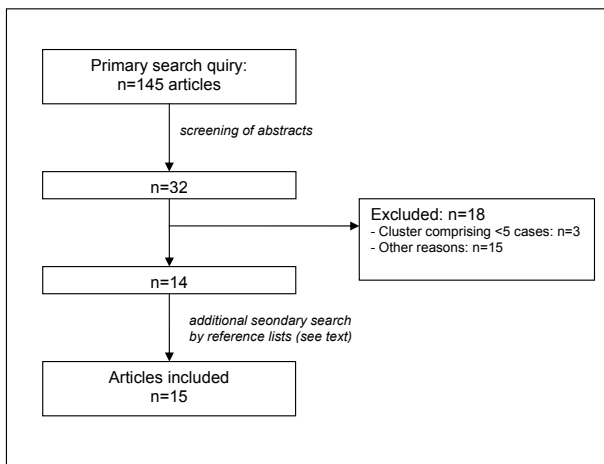


Table 1. Included *Pneumocystis* outbreaks in kidney transplant recipients 1980-2010.

No., 1st Author (ref)	Publication (year)	Geographic location	Index case (year)	N	<1year post transplantation (%)	Risk analysis (case-control)	Genotyping of <i>Pneumocystis</i>	Reported mortality (%)
1- Hardy [22]	1984	Pittsburg, USA	1982	14	86	Yes	No	21.4
2- Santiago-Delpin [3]	1988	San Juan, Puerto Rico	1985	11	NA	No	No	45.5
3- Talseth [4]	1988	Oslo, Norway	1985	14	100	No	No	50.0
4- Bensousan [8]	1990	Brest, France	1990	6	NA	No	No	NA
5- Chave [7]	1991	Lausanne Switzerland	1988	5	40	No	No	NA
6- Branten [9]	1995	Nijmegen, Netherlands	1991	28	NA	Yes	No	21.4
7- Hennequin [10]	1995	Paris, France	1995	7	86	No	No	42.9
8- Lufft [11]	1996	Hannover, Germany	1993	7	NA	No	No	0
9- Olsson [50]	2001	Solna, Sweden	1987/1988	5+7 [†]	NA	No	Yes	NA
10- Rabodorina [13]	2004	Lyon, France	1994	10	50	No	Yes	10.0
11- de Boer [2]	2007	Leiden, Netherlands	2005	22	50	No	Yes	4.5
12- Schmoldt [15]	2008	Munchen, Germany	2006	16	94	No	Yes	25.0
13- Yazaki [14]	2009	Nagoya, Japan	2004	27	41	No	Yes	3.7
14- Arichi [16]	2009	Hyogo, Japan	2007	9	44	Yes	No	33.3
15- Gianelli [12]	2009	Zurich, Switzerland	2006	20	55	No	Yes	15.0

Legend: NA denotes that information was not available; † 2 separate clusters were described.

Results and Discussion

Descriptive epidemiology

A total of 16 outbreaks, described in 15 articles, were identified and comprised a median number of 12 cases, with a range up to 28 cases. In the first reports of clusters of PCP in kidney transplant units in the 1980s, the rise of PCP incidence was linked to the rapidly expanding solid organ transplanted population and the introduction of Cyclosporine as a maintenance drug to prevent graft rejection [3, 21]. Overlapping hospitalization of PCP cases was already noted but absence of molecular methods only allowed speculation about the possibility of interhuman transmission [3, 4, 22]. In one study a case-control investigation strongly indicated transmission of PCP from HIV-infected patients to kidney transplant recipients [7]. At the time, this observation was seen as support for the hypothesis that the overall rising incidence of PCP due to the HIV epidemic influenced the risk for PCP in other immunocompromised populations [23]. More extended mapping of potential interhuman transmission occasions was performed in later outbreak studies. These detailed descriptions all demonstrated the high probability of frequent contact between kidney transplant recipients who developed

PCP. However, this observation may be inherent also to the post transplant state in which patients have to submit to frequent hospital visits. Interestingly, none of the studies report a coincidental increase in PCP incidence in other immunocompromised populations e.g. liver or lung transplant recipients at the same institution. The epidemiological data of the larger outbreaks ($n > 15$, all occurring after 1990) show bell shaped incidence curves, suggesting a common origin resulting from either interhuman transmission or an environmental source.

A period of preceding years with very low prevalence ($< 2\%$) of PCP in the kidney transplant population was reported in 75% of the outbreaks. As discussed by many of the authors, this low incidence of PCP had prompted local transplant committees to weigh Trimethoprim-Sulfamethoxazole side effects against overall morbidity and mortality of PCP. In the respective institutions this resulted in a policy endorsing the absence of PCP chemoprophylaxis post kidney transplantation. Furthermore, avoidance of placement of a patient with PCP next to other immunocompromised patients - as currently recommended by the Centers for Disease Control and Prevention (CDC) - was not routinely practiced at the time in any of the outbreak settings [24].

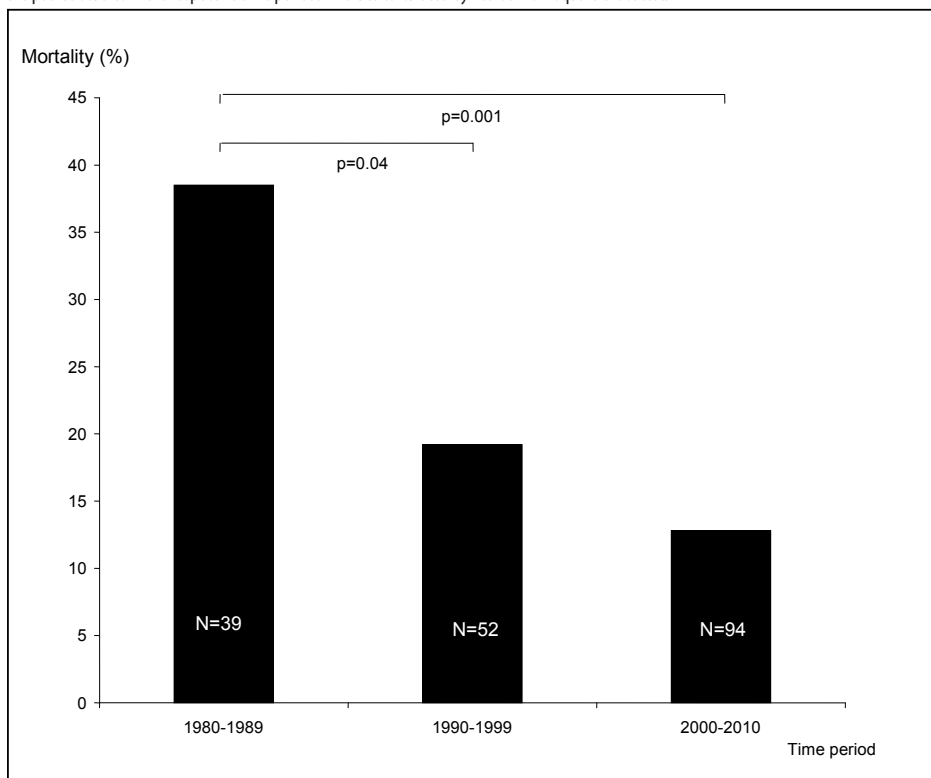
Mortality

Over time, mortality rates in kidney transplant recipients during outbreaks declined from a weighted average of 38% in the 1980s ($n=3$ studies) to 19% in the 1990s ($n=4$ studies) and 13% in the first decade of this century ($n=5$ studies). For 3 studies no mortality data were available. The trend in declining mortality was significant between the first and the last two decades (figure 2). The mortality in HIV-negative immunocompromised patients with PCP has been reported to be relatively high as compared to HIV-positive patients with PCP. In autopsy studies of patients with PCP, the number of Pneumocysts was found to be relatively lower in immunocompromised patients without HIV [25]. In contrast, inflammation was found to be more extensive ref. These observations indicate that not *P. jirovecii* burden but the intensity of the evoked inflammatory reaction determines the severity of disease and the risk of fatal outcome. A number of trials published in the early 1990s demonstrated the beneficial effects of steroids in the treatment of PCP [26-28]. The implementation of conjunct steroid treatment probably accounts for the observed decrease in PCP associated mortality after 1990. In addition, early diagnosis and the availability of high quality intensive care facilities are other important factors that probably attribute to a declining mortality [29].

Clinical risk factors

Only 3 out of the 15 outbreaks explored the potential risk factors by a retrospective case-control investigation. Up to date, only two other retrospective case-control studies that specifically focused on identification of risk factors for PCP in kidney transplant recipients

Figure 2. Histogram showing average *Pneumocystis pneumonia* associated mortality per decade; N represents the total number of cases from the pooled studies in the respective time period. P-values calculated by Pearson-chi-square exact test.



Legend: N represents the total number of cases from pooled studies per time period. P-values were calculated by Pearson-chi-square test.

not receiving any prophylaxis, were published [30, 31]. Since the included patients in these studies were isolated cases, they were not primarily included for the purpose of this review. Table 2 shows the major findings of the risk factor analyses in two non-outbreak and three outbreak investigations. Patients were most at risk in the first year post transplantation. The weighted average percentage of cases diagnosed within the first year post transplantation was 63% (range: 40% to 100% of cases).

Rejection treatment

A higher frequency of treatment for rejection prior to development of PCP was reported from both non-outbreak studies. In the complete series of outbreak studies any previous treatment for rejection, i.e. high dose steroids or ATG (anti-thymocyte globulin) was present in 22% to 100% of cases (median 50%). Data from 4 outbreak studies specifically reported the use of ATG or OKT3 globulins as rejection treatment in 20 to 39% of cases as compared to 41% and 73% in the two non-outbreak studies. Insufficient data was available to assess

Table 2. Potential individual risk factors for *Pneumocystis pneumonia* in outbreak and non-outbreak settings.

Study: 1 st author, year	Non-outbreak studies		Outbreak studies		
	Arend ^[30] 1995	Radisic ^[31] 2003	Hardy ^[22] 1984	Branten ^[9] 1995	Arichi ^[16] 2009
No. included cases/controls	15/95	17/34	14/26	28/27	10/431
Mean age: (years)					
cases	48.8	39.7	40.7	50*	46.9*
controls	43.3	35.4	NA ^β	38	34.0
Treatment for rejection (any): n (%)					
cases	13 (87)*	17 (100)*	10 (71)	13 (46)	NA
controls	57 (60)	26 (76)	NA	11 (41)	NA
ATG/OKT3 as rejection treatment: n (%)					
cases	11 (73)*	7 (41)*	NA	11 (39)	2 (20)
controls	30 (32)	3 (9)	NA	6 (22)	29 (7)
CMV infection: n (%)					
cases	8 (53)*	9 (53)*	14 (100)*	5 (18)	NA
controls	15 (19†)	8 (24)	13 (65‡)	4 (15)	NA

Legend: NA denotes that information was not available; †: of 80 controls for whom data about CMV was available; ‡: of 20 cases for whom data about CMV was available. β: cases and controls were matched for age in this study. *: significant difference between cases and controls ($p < 0.05$).

the role of basiliximab or other immunotherapy's administered at time of transplantation to prevent rejection.

CMV infection

Concurrent CMV infection was associated with development of PCP in both non-outbreak studies as well as in 2 of the 3 case-control investigations within outbreak studies. A high incidence of CMV infection - either reactivation or primary infection - coinciding with PCP was reported in 7 out of the total of 15 outbreak studies. The weighted average percentage of cases with concurrent CMV replication was 63% (range 18% to 100%). Comparisons of these percentages are flawed by changes in the diagnostic methods as well as definition of CMV infection in kidney and other solid organ transplant populations. In addition, new screening and prevention strategies for CMV disease have been implemented over time [32]. It remained unclear whether the association of CMV infection with PCP is due to the underlying gap in T-cell function, putting patients at risk for both infections, or due to the suppressive effect of CMV replication on T-cell function itself. Several clinical studies and in-vitro experiments indicate that CMV has a negative effect on the cellular immune response [33].

Immune suppressive regimen

The occurrence of PCP in renal transplant recipients was directly linked to the immunosuppressive regimen in 4 out of the 15 studies [3, 11, 16, 22]. In two studies the PCP outbreak coincided with the introduction of Cyclosporine A as part of the immunosuppressive therapy [3, 22]. The influence of Cyclosporine A on the risk for PCP was additionally suggested in the publication of case series from several large transplantation centers in the 1980s [21, 34]. However, contradicting observations were reported from case series as well as in one of the outbreak studies [11, 35]. In the case control investigations (of both outbreak and non-outbreak studies) Cyclosporine A based immune suppression was found not to be associated with increased risk for PCP. Experimental studies in rats had previously demonstrated a protective effect of the purine antagonist Mofetyl Mycophenolate against PCP [36]. However, the clinical relevance of this finding was never confirmed through comparative research in humans [16;37;38]. In the multivariate analysis performed in the outbreak study performed by Arichi et al., the use of Mofetyl Mycophenolate was even associated with increased risk for PCP [16].

Molecular epidemiology

In 6 of the 15 included outbreak studies a molecular analysis of the *P. jirovecii* organisms was undertaken to investigate the possibility of interhuman transmission. Table 3 shows the genotyping results of the six PCP outbreak studies among kidney transplant recipients. In 5 of the outbreaks one predominant or a single strain was identified.

Over 14 unique gene loci have been evaluated for the purpose of genotyping applications [37]. The original approach by sequencing of the mitochondrial large subunit (mt LSU) RNA in conjunction with single stranded confirmation polymorphism (SSCP), was used in only 1 of the included studies. After this time newer methods were preferred. Multilocus sequence

Table 3. Genotyping of *Pneumocystis* organisms in 7 outbreaks of *Pneumocystis* pneumonia in kidney transplant recipients.

Study No.	Method	No. of cases included in genotyping analysis / total No. of cases	Genotyping result	Reference group included in the analysis
-9-	Mt LSU RNA +SSCP	3 / 5	Different strains	No
		7 / 7	2 pairs of cases with identical strains	No
-10-	MLST	9 / 10	6 out of 9 strains identical	Yes
-11-	ITS1+ITS2	16 / 22	12 out of 16 strains identical	Yes
-12-	MLST	16 / 16	all strains identical	Yes
-13-	ITS1+ITS2	8 / 27	all strains identical	Yes
-15-	MLST	7 / 7	all strains identical	Yes

Legend: Mt-LSU denotes mitochondrial large subunit ribonucleic acid; MLST: multilocus sequence typing; ITS: internal transcribed spacer region; SSCP: single strand confirmation polymorphism.

typing (MLST) described by Hauser et al. [38], probably has become the most frequently used method and was applied in 3 of the outbreak studies. ITS genotyping, described by Lee et al. [39], was used in 2 of the 6 outbreak studies that applied genotyping methods as well as in several cross sectional epidemiological studies investigating the geographic distribution of *P. jirovecii* genotypes [40, 41]. Interpretation of *P. jirovecii* genotyping results in any outbreak of *Pneumocystis* needs to be performed with care. Several lines of evidence now strongly suggest that infection with *P. jirovecii* occurs through airborne transmission from either patients with overt PCP or individuals – either healthy or immunocompromised – colonized with *P. jirovecii*. This model implicates that genotyping results obtained from an outbreak must not only be linked within the outbreak itself, but should be held also against the background of the circulating genotypes in the population. Background information on circulating strains is necessary to determine whether the outbreak is more likely due to patient-to-patient transmission or increased random transmission from colonized individuals. Secondly, colonization and infection with more than one *P. jirovecii* genotype has been described. The validity of the genotyping methods to detect these double or triple configuration of *Pneumocystis* strains involved is yet unknown and may further complicate interpretation of the genotyping results.

An interpretation of the genotyping results of the outbreak against the background of circulating strains was performed in 5 out of the 6 studies in which genotyping methods were applied. Although reference groups were relatively small ($n < 50$), the genotypes of strains from the control groups were found to be different from those detected in the respective outbreaks. Difficulties in interpretation of the genotyping results due to presence of multiple strains were not reported as a major problem. Variation in virulence between individual *Pneumocystis* strains may have contributed to the genesis of PCP outbreaks. Thus far, investigations failed to indicate such a possible relationship [42].

Environmental investigations

In 2 out of the 15 outbreak studies a local environmental investigation was performed in the hospital. Yazaki et al. [14] performed two sets of environmental surveys using 30-40 swabs in areas visited by affected patients. *P. jirovecii* DNA was found in outpatients consulting rooms. However, the finding could be interpreted as a consequence of the observed outbreak as well as a link to the (environmental) cause. In a study by our group, air samplers were used to collect air specimens during the aftermath of the observed outbreak [2]. From the air filters no *Pneumocystis jirovecii* DNA could be extracted. Though limited by the lack of validated sampling methods, the search for a specific environmental source, during outbreaks and in separate investigations, practically remained without meaningful results [43, 44]. Recently, Choukri et al. published the first report of detection of dispersion of *P. jirovecii* in the air surrounding PCP patients [45]. In this study it was demonstrated that a significant relation existed between the amount of detected *P. jirovecii* DNA and distance from the patient diagnosed with PCP. This

finding further supports the hypothesis that airborne transmission occurs during contact between patients with *Pneumocystis* pneumonia and individuals at risk [45].

Climatic influence

A total of 10 out of the 15 studies contained data about the timing of the cases throughout the year. The timing of peak incidence occurred during winter in 4, during spring in 1 and in summer or early fall in 5 out of 10 studies. The time of diagnosis of the index case was not associated with any month or season. The majority of studies were reported from geographic locations with moderate climate in coastal areas. In epidemiological studies investigating the possible association between overall PCP incidence and climatologic factors from the UK and Spain, a positive correlation was found between PCP incidence and colder months [46-48]. In contrast to these findings, a recent study from Germany, found that higher incidences of PCP were associated with the summer period by using four different climatic factors and the season as variables in a multivariate statistical model [49]. A possible explanation for these different observations may be that other more important factors linked to climate, e.g. human behavior, outweigh the influence of single climatic factors with regard to *Pneumocystis* transmission. As suggested by the study reported by Santiago-Delpin [3], the occurrence of *Pneumocystis* is not restricted to temperate climates only.

Summary and Conclusions

Over time, the descriptive epidemiological and genotyping data of the 15 PCP outbreak studies increasingly pointed to a common source, either environmental or human. This is in contrast to the previous concept of reactivation of *Pneumocystis* in the immunocompromised host, but concurs with the current hypothesis of acquisition of *Pneumocystis* via individuals that are carriers of *P. jirovecii* or that suffer from PCP. Though limited by available methods, the search for a specific environmental source during outbreaks and in separate investigations remained without result. PCP associated mortality rates significantly decreased from a weighted average of 38% before 1990 to less than 20% in the past two decades. Clinical risk factors for PCP during outbreaks were largely similar to non-outbreak settings. Alterations in immune suppressive regimens were at first highly suspected, but never proven to be a major factor in the cause of an outbreak. Treatment for rejection, CMV infection and (potentially) older age were the most important individual risk factors. During the past decades changes occurred with regard to most of these factors. The age limit for solid organ transplantation gradually shifted upwards and more advanced T-cell specific compounds for maintenance immune suppression and treatment as well as prevention of rejection became available. This could have shifted the ratio of kidney transplant recipients at risk versus not at risk for

development of PCP within the kidney transplant population. As a consequence, this would at least facilitate the initiation and propagation of an outbreak. Furthermore, two other important factors permitted the development of the PCP outbreaks. First, the lack of application of droplet isolation measures during hospitalization of kidney transplant recipients with PCP probably increased the exposure of the rest of the population at risk. More importantly, the risk for development of PCP was not negated by the use of chemoprophylaxis for PCP in any of the reported outbreaks settings.

The studies of PCP outbreaks in kidney transplant recipients provide important data that have contributed to the understanding of the mode of transmission and epidemiology of *Pneumocystis*. The discovery of the linkage of each species of *Pneumocystis* to a specific mammalian host and the phenomenon of common asymptomatic carriage in the airways of both healthy and immunocompromised hosts further supports the hypothesis that the human population forms the primary – if not the only - source.

With regard to the data presented in this review, it must be concluded that although PCP incidence in kidney transplant recipients may be low for a prolonged period of time, a chemoprophylactic strategy needs to be implied to prevent both incidental cases and PCP outbreaks. Furthermore, isolation measures must be installed during hospitalization of patients with PCP to prevent transmission to other individuals at risk.

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