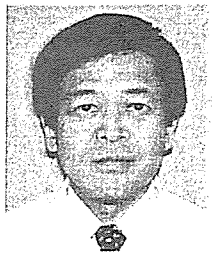
地域支援ネットワーク構築の新しい流れ

——地域ネットワークの現状と将来展望

Local networks for supporting nosocomial infection control in Japan



切替照雄(写真)　倉辻忠俊

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◎各自治体と厚生労働省による共同モデル事業として，平成16年(2004)度から全国10道県と1政令都市の11地域で院内感染対策を支援するための院内感染地域支援ネットワークが設置され，支援活動を開始した．地域支援ネットワークでは院内感染相談業務と普及啓発事業を中心に，状況に応じてさまざまな支援活動を実施している．これらの支援活動を通じて“院内感染対策地域支援ネットワーク活動は，地域医療におけるインフェクションコントロールチーム活動である”という方向に活動が定まってきた．また，院内感染対策地域支援ネットワークが，地域全体の中小規模病院や老健施設などにおけるインフェクションコントロールチームの活動を肩代りできる可能性が示されてきた．ネットワーク活動は地域の実情によって，ネットワークの運営の中心となる支援委員の構成や活動の主体となる組織が異なるなどの多様性，地域差がみられてきた．

Key word：院内感染対策地域支援ネットワーク，院内感染対策，インフェクションコントロールチーム

背景

わが国の医療機関における院内感染対策はこの20年で著しく改善したと多くの医療従事者は考えているであろう．実際，今日，ほとんどの病院で院内感染対策委員会が設置され，月1回程度の頻度で委員会が開催されている[1]．79%の病院では感染制御医師(infection control doctor：ICD)や感染管理看護師(infection control nurse：ICN)などで構成される実務担当者が任命されている[2]．大規模な病院では，これら実務担当者がインフェクションコントロールチーム(infection control team)として院内感染対策の実務を担当している．環境感染学会などの院内感染対策関連の学会，院内感染対策に関する講習会では，これらの実務担当者が積極的に参加し，まさに大盛況である．

しかし一方では，感染症の専門家が少ない，あるいは専門家がいても日常業務に忙殺されている中小規模の医療・介護施設が多く存在している．これらの施設では専任の感染管理師がいないため，十分な感染対策が実施されないことが危惧されている．このような医療施設に対して，地域で支援するシステムが必要であることは明らかである．

平成15年(2003)に厚生労働省は，わが国におけるあらたな院内感染対策のグランドデザイン，および医療機関，自治体，国，関係団体・学会がそれぞれの立場で取組むべき事項をまとめた『院内感染対策有識者会議報告書—今後の院内感染対策のあり方について』を公表した[2]．このなかで，自治体に対しては“自治体(都道府県等)を単位として院内感染地域支援ネットワークが組織され，日常的に医療機関からの院内感染対策に関する相談に応じるとともに，院内感染の大規模な集団発生や対策を講じているにもかかわらずその発生が継続する場合等，若しくは発生が疑われる場合に，医療機関に対し速やかに相談に応じ，助言を行う

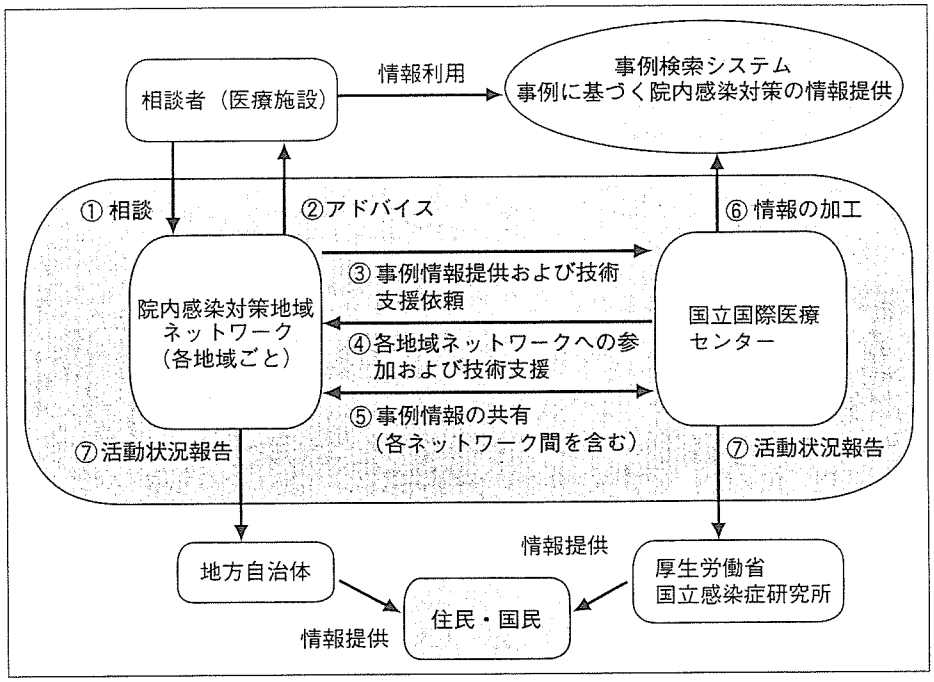

図 1　院内感染対策相談事例事業全体の流れ

体制が構築されている”ことを，国に対しては“各地の院内感染地域支援ネットワーク及び行政機関等に集まった院内感染事例を集積したデータベースが構築されており，それが地域の医療機関や自治体に広く利用可能なものとなり，情報や経験の共有に活かされている”ことを感染対策の将来像として掲げている．このような背景で，平成16年(2004)から院内感染地域支援ネットワークがモデル事業として始まった．

ネットワーク活動の目的

院内感染対策地域支援ネットワークは，地域の専門家から構成されるネットワークを構築して地域における支援体制の整備をはかり，規模の大小を問わず医療機関などが速やかに相談や助言を受けることができる体制を整備すること，さらに各ネットワーク間でたがいに情報交換をして，それぞれの支援活動の質を高めていくことを目的として事業活動を実施している[3]．

北海道，青森県，埼玉県，静岡県，富山県，岐阜県，滋賀県，岡山県，香川県，北九州市および鹿児島県の11地域で院内感染対策を支援するための院内感染地域支援ネットワークが設置されている．このネットワークでは，院内感染対策の相談業務を通して地域医療機関で発生した事例を収集，解析することになるが，このうち重要な事例については匿名化し，国へ情報提供するための方法についてシステム化をはかる必要がある．また，国において重要な事例の収集，解析，評価を行ったうえで地域などに還元することにより，これらの事例の経験を共有し，同様のあるいは類似した原因による院内感染の発生を防止すべく今後の対策に生かすことができる．このように地域のネットワークなどから国へ情報提供を行うためのシステムと，国における院内感染事例データベースを構築し，医療施設への情報還元方法を考案し，さらに国民に情報を公開していかなければいけない(図1)．しかし，なんといっても重要なのが各地域の支援ネットワーク活動，とくに地域の医療施設が気軽に相談できるシステムを構築することである．

ネットワーク活動形態

モデル地域として，全国9道県と1政令都市が院内感染支援ネットワークを平成16年(2004)1～12月に開設している(岐阜県は平成18年度後半から参加したため，岐阜県は除かれた)．ネットワーク活動は，地域の実情によってさまざまな活

動形態となっている．とくに大きな違いは，支援ネットワーク活動拠点，すなわち実際の活動の中心となる組織がどこなのか，それとネットワークの運営の中心となる支援委員の構成がどのようになっているのかの2点である．

支援ネットワークに参加している組織として地方衛生研究所などの地方自治体，医師会や病院協会，大学医学部や地域の中核病院などがあり，これらの組織がたがいに協力しあってネットワークを構築している．しかし実際は，それぞれの地域によってネットワーク活動の中心となる組織が異なっている．

ネットワークの支援委員の構成も，それぞれの地域で異なっている．地域の医療現場でインフェクションコントロールチーム活動を実際に行っている専門家で構成されている場合，医師会，歯科医師会，看護協会，保健所，行政，大学の代表者から構成されている場合，大学や地域中核病院の感染症科や感染症制御部が活動の中心となっている場合などがあった．

それぞれの地域で，状況に適したネットワークを構築している．医師会や病院協会が活動の中心となっている地域は5地域あった．地域によって活動内容に違いがあり，非常に熱心な地域ではネットワークと医療現場との距離感が少なく，ネットワークの裾野の広がりがある．今後のネットワーク支援活動のモデルになるような活動をしている．

大学などが活動の中心となっている地域が5つあった．そのうち活動が盛んな地域では，大学などが地域内の各医療施設を取り込んだネットワーク活動を展開していた．ネットワーク活動に大学が加わることも重要である．ただし問題点として，どれだけ裾野を広くできるのかに課題が残る．大学がネットワーク活動の中心となる場合では，地域の医療現場の専門家が広く参加する仕組みを取り入れることが肝心であろう．また，地域内に院内感染専門家が比較的少ない地域ではネットワークの支援委員の人選が難しく，県外の大学に全面的に活動を委託している地域もある．このような地域では，今後専門家をどのように育成していくのかが重要である．

地方衛生研究所が活動の中心となっている地域が1地域あった．この地域ではインターネットをうまく利用して相談業務と啓発業務活動を行っている．今後は，感染症専門家および医療機関のインフェクションコントロールチームのメンバーから登録メンバーを募り，裾野を広げる方向で活動を進めている．

以下に活動形態についてまとめる．

① 相談窓口設置場所……大学附属病院，病院協会，県立病院内や医師会などさまざまである．

② 相談形式……電話が埼玉県，電話とファックスが静岡県，メールが岡山県，電話・ファックス・メールが滋賀県，香川県と北九州市，ファクスとメールが鹿児島県，ウェブ掲示版(会員制)が北海道であった．とくに北海道ではウェブ上で相談者と相談対応者が，セキュリティー強化された電子掲示板に書き込む方式で随時行われている．

③ 窓口業務時間，窓口担当者の有無……電話の相談担当者を配置しているところでは業務時間を設置していた．

④ 相談対応者……北海道は5名の相談対応者と48名の相談者(アドバイザー)，青森県は16名，埼玉県5名，富山県17名，静岡県10名(別に窓口担当者1名)，滋賀県3名(うち窓口担当者1名)，香川県4名，岡山県2名，鹿児島県5名(うち窓口担当者1名)であった．

⑤ 相談件数……平成17年度の相談件数は合計250件であった．昨年度113件と飛躍的に増加した．各ネットワークの相談件数は0～50件とばらつきがあった．

活動内容

ネットワークの活動内容は，現時点では院内感染相談と普及啓発事業が中心である．普及啓発事業は，パンフレット，書籍，セミナーに加えてホームページを作成している．どの地域もネットワーク活動自体の広報に力を入れ，とくに広報活動も含めたセミナーなどの啓発事業を重点的に実施していた．ネットワーク主催のセミナーや講習会に

は毎回たいへん多くの医療関係者が聴衆として集まっていて，医療現場における院内感染対策への関心の高さを改めて感じさせた．また，いわゆる“困ったこと”に代表される院内感染事例相談が，活動のなかで重要性を徐々に増している．もっとも多い相談内容(相談項目は内容によって複数のカテゴリーに分類)は消毒法に関する質問で，36件であった．ついで，微生物検査に関して25件，感染対策マニュアルに関する質問18件，個別管理に関する質問14件，環境管理に関する質問11件，検体曝露事例14件などであった．

今後のネットワーク事業のモデルとなる地域

たいへん成功しているモデル地域の1例(静岡県)では病院協会にネットワークの窓口を設置している．地域内の10カ所の中核病院でそれぞれインフェクションコントロールチームの中核メンバーとなっている10名の医師が，支援委員として活動をしている．活動の中心は院内感染相談業務と普及啓発事業である．普及啓発事業としては，中小規模の病院および高齢者施設を対象にした教育セミナー(参加152施設)，冊子などの資料配付(1,700部，421施設)，相談事例(Q & A)のホームページ掲載などをしている．このQ & Aは非常に質の高い回答が掲載されており，たいへん参考になる．この地域の活動は，院内感染支援ネットワークが地域全体の中小規模病院などの医療施設のインフェクションコントロールチーム活動を肩代りしていることを実感できる．なぜ，このモデル地域が短期間にたいへん有効なネットワーク事業を展開することが可能になったのか，いくつかの理由があるであろう．①すべての支援委員が医療現場で実際に活躍している専門家であること，②この地域では多くの院内感染対策の専門家がすでに活躍していたこと，③支援委員が地域内の各地方から集まり地域的な偏在がないこと，④地域の病院協会の大きな支援があること，などではないかと考えられる．いずれにせよ，地域全体の院内感染対策の裾野を広げることが地域支援ネットワークの質の向上につながると考えられる．

まとめ

11モデル地域での院内感染対策を支援するための院内感染地域支援ネットワークが設置され，支援活動が開始された．“院内感染対策地域支援ネットワーク活動は，地域医療におけるインフェクションコントロールチーム活動である”という方向に活動が定まってきた．今回のモデル事業で明らかになってきたことは，院内感染対策地域支援ネットワークが地域全体の中小規模病院や老健施設などにおけるインフェクションコントロールチームの活動を肩代りできることである．

文献/URL

1) 大久保　憲：平成14年度厚生労働科学特別研究事業「わが国の院内感染対策の整備状況を把握するための研究」報告書．2003.
2) 厚生労働省：院内感染対策有識者会議報告書—今後の院内感染対策のあり方について．2003.(http://www.mhlw.go.jp/shingi/2005/01/s0113-6b.html)
3) 倉辻忠俊：平成16年度厚生労働省科学医療技術評価総合研究事業「院内感染地域支援ネットワーク及び相談体制の改善・普及や，データベースおよびバックアップ体制の構築に関する研究．2005.

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Outbreaks of Multidrug-Resistant *Pseudomonas aeruginosa* in Community Hospitals in Japan[▽]

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We previously reported an outbreak in a neurosurgery ward of catheter-associated urinary tract infection with multidrug-resistant (MDR) *Pseudomonas aeruginosa* strain IMCJ2.S1, carrying the 6′-*N*-aminoglycoside acetyltransferase gene [*aac(6′)-Iae*]. For further epidemiologic studies, 214 clinical isolates of MDR *P. aeruginosa* showing resistance to imipenem (MIC ≥ 16 μg/ml), amikacin (MIC ≥ 64 μg/ml), and ciprofloxacin (MIC ≥ 4 μg/ml) were collected from 13 hospitals in the same prefecture in Japan. We also collected 70 clinical isolates of *P. aeruginosa* that were sensitive to one or more of these antibiotics and compared their characteristics with those of the MDR *P. aeruginosa* isolates. Of the 214 MDR *P. aeruginosa* isolates, 212 (99%) were serotype O11. We developed a loop-mediated isothermal amplification (LAMP) assay and a slide agglutination test for detection of the *aac(6′)-Iae* gene and the AAC(6′)-Iae protein, respectively. Of the 212 MDR *P. aeruginosa* isolates, 212 (100%) and 207 (98%) were positive in the LAMP assay and in the agglutination test, respectively. Mutations of *gyrA* and *parC* genes resulting in amino acid substitutions were detected in 213 of the 214 MDR *P. aeruginosa* isolates (99%). Of the 214 MDR *P. aeruginosa* isolates, 212 showed pulsed-field gel electrophoresis patterns with ≥70% similarity to that of IMCJ2.S1 and 83 showed a pattern identical to that of IMCJ2.S1, indicating that clonal expansion of MDR *P. aeruginosa* occurred in community hospitals in this area. The methods developed in this study to detect *aac(6′)-Iae* were rapid and effective in diagnosing infections caused by various MDR *P. aeruginosa* clones.

Pseudomonas aeruginosa causes nosocomial infections as a result of its ubiquitous nature, ability to survive in moist environments, and resistance to many antibiotics and antiseptics. A serious problem is the emergence of multidrug-resistant (MDR) *P. aeruginosa* strains resistant to β-lactams, aminoglycosides, and quinolones (34, 39, 46). Although intrinsically sensitive to β-lactams (e.g., ceftazidime [CAZ] and imipenem [IPM]), aminoglycosides (e.g., amikacin [AMK] and tobramycin), and fluoroquinolones (e.g., ciprofloxacin [CIP] and ofloxacin [OFX]), *P. aeruginosa* resistant to these antibiotics has emerged and is widespread (34, 39, 46).

We previously reported a nosocomial outbreak of catheter-associated urinary tract infection involving new MDR *P. aeruginosa* strain IMCJ2.S1, which occurred in a neurosurgery ward of a hospital located in the Tohoku area of Japan (46). This strain showed broad-spectrum resistance to aminoglycosides, β-lactams, fluoroquinolones, tetracyclines, sulfonamide, and chlorhexidine. We found that IMCJ2.S1 harbored a novel class 1 integron, In113, containing an array of three gene cassettes of the metallo-β-lactamase (MBL) bla_{IMP-1} gene, aminoglycoside 6′-acetyltransferase *aac(6′)-Iae* gene, and aminoglycoside 3′-adenylyltransferase *aadA1* gene (46). This strain possessed mutations of the *gyrA* (83Thr→Ile) and *parC* (87Ser→Leu) genes involving amino acid substitutions, resulting in high-level resistance to fluoroquinolones.

In the geographic area where the MDR *P. aeruginosa* outbreak occurred (46), hospitals and a commercial clinical laboratory were surveyed for similar organisms. Because 99% of the MDR *P. aeruginosa* isolates analyzed were found to harbor the *aac(6′)-Iae* gene, we developed a loop-mediated isothermal amplification (LAMP) assay (31) and a slide agglutination assay to detect the *aac(6′)-Iae* gene and AAC(6′)-Iae protein, respectively. These methods were evaluated for their usefulness in detecting new MDR *P. aeruginosa* strains.

MATERIALS AND METHODS

Bacterial strains. Criteria for multidrug resistance of *P. aeruginosa* were in accordance with the Law Concerning the Prevention of Infections and Medical Care for Patients with Infections of the Japanese Ministry of Health, Labor, and Welfare; the criteria are resistance to imipenem (MIC ≥ 16 μg/ml), amikacin (MIC ≥ 64 μg/ml), and ciprofloxacin (MIC ≥ 4 μg/ml). The criterion for amikacin resistance (MIC ≥ 64 μg/ml) was different from that of a guideline of the Clinical and Laboratory Standards Institute (MIC ≥ 32 μg/ml) (4). Two

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hundred eighty-four clinical isolates of *P. aeruginosa* were obtained from 284 inpatients in 13 hospitals in Japan during the period October 2003 to September 2004; 214 isolates were MDR, and 70 were non-MDR. Information regarding the origins of the specimens was available for 99 of the 214 MDR isolates: 72 (73%) were from urine specimens, 18 (18%) were from respiratory tract specimens, 5 (5%) were from feces, 2 (2%) were from catheter tips, and 2 (2%) were from wounds. Of the 72 isolates from urine, 55 were from patients with urinary catheters. All *P. aeruginosa* isolates were originally identified by the submitting laboratories. Isolates that did not have typical characteristics (pigment and colony morphology) for *P. aeruginosa* were analyzed biochemically with an API 20NE kit (API-bioMerieux, La Balme les Grottes, France) to confirm identity as *P. aeruginosa*. *P. aeruginosa* M207 possessing bla_{IMP-1}, *P. aeruginosa* NCB326 possessing bla_{IMP-2}, and *Acinetobacter baumannii* NCB0211-439 possessing bla_{VIM-2} were provided by Y. Arakawa (National Institute of Infectious Diseases, Tokyo, Japan). *Escherichia coli* strain TOP10 (Invitrogen Corp., Carlsbad, CA) was used as the host for recombinant plasmids.

Serotyping. The O serotypes of the isolates were determined with a slide agglutination test kit containing three polyvalent antisera and 14 monovalent antisera (Denka Seiken Co., Tokyo, Japan). The kit was not in conformity with the International Antigenic Typing Scheme (IATS) (26) and was not applicable to some O types in the IATS. Therefore, we applied the standard classification of O types from A to N proposed by the Serotyping Committee for the Japan *Pseudomonas aeruginosa* Society (12).

Antimicrobial susceptibility. We obtained AMK and IPM from Banyu Pharmaceutical Co. (Tokyo, Japan), arbekacin [1-*N*-(*S*)-4-amino-2-hydroxybutyl dibekacin; ABK] from Meiji Seika Kaisha, Ltd. (Tokyo, Japan), aztreonam (AZL) from Eizai (Tokyo, Japan), CAZ from GlaxoSmithKline K. K. (Tokyo, Japan), CIP and OFX from Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan), doripenem (DRPM) from Shionogi & Co., Ltd. (Osaka, Japan), gentamicin (GEN) and streptomycin (STR) from Nacalai Tesque, Inc. (Kyoto, Japan), meropenem (MEM) from Sumitomo Pharmaceutical Co., Ltd. (Osaka, Japan), piperacillin (PIP) and piperacillin-tazobactam (TZP) from Tomiyama Pure Chemical Industries, Ltd. (Tokyo, Japan), and polymyxin B (PL-B) from Sigma-Aldrich (St. Louis, MO). Arbekacin is an aminoglycoside antibiotic and has been used for the treatment of methicillin-resistant *Staphylococcus aureus* infections in Japan (51). Values for MICs at which 50% of isolates were inhibited (MIC_{50}) and MIC_{90} were determined by the microdilution method according to the Clinical Laboratory Standards Institute (CLSI, formally NCCLS; standard M7-A6) (4) except for ABK, PL-B, and STR, for which breakpoints (≥4 μg/ml) were obtained from the published data (16, 30, 46).

Screening for MBL-producing *P. aeruginosa*. *P. aeruginosa* isolates were screened for the presence of MBL by a double-disk synergy test with disks containing sodium mercaptoacetic acid, according to the method of Arakawa et al. (2).

Immunologic detection of AAC(6′)-Iae. To detect AAC(6′)-Iae produced by *P. aeruginosa*, we developed a new method with AAC(6′)-Iae antibody-conjugated beads. Recombinant AAC(6′)-Iae was purified as reported previously (46) and used for immunization of Japanese white rabbits. Antibody against AAC(6′)-Iae was affinity purified from rabbit antisera with an *N*-hydroxysuccinimide–Sepharose column (Amersham Pharmacia Biotech, Piscataway, NJ) conjugated to recombinant AAC(6′)-Iae. Purified antibody was coupled to Polybead carboxylated microspheres (2.022 μm in diameter; Polysciences, Inc., Warrington, PA) according to the manufacturer's instructions. Antibody-conjugated beads were suspended at 2.5% (vol/vol) in 0.1 M phosphate buffer (pH 7.4) containing 0.1% sodium azide. Agglutination tests were performed with *P. aeruginosa* isolates grown on *N*-acetyl-L-cysteine agar medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). Bacterial cells suspended in distilled water were mixed with the antibody-conjugated beads. To confirm the specificity of the agglutination test, *P. aeruginosa* isolates were analyzed by conventional Western blotting with AAC(6′)-Iae antibody.

PCR of class 1 integrons. Class 1 integrons responsible for multidrug resistance in *P. aeruginosa* (21, 34, 46) were detected and characterized by PCR as described previously (24). Primer pairs designed to amplify the gene cassette of In113 (46) and three primer pairs specific for bla_{IMP-1}, bla_{IMP-2}, and bla_{VIM-2} (47) were used. Positive controls were *P. aeruginosa* IMCJ2.S1 for class 1 integron In113, *P. aeruginosa* M207 for bla_{IMP-1}, *P. aeruginosa* NCB326 for bla_{IMP-2}, and *A. baumannii* NCB0211-439 for bla_{VIM-2}. PCR was performed with a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA). Genomic DNA was extracted as described by Sambrook et al. (44). When unexpected sizes of PCR products were obtained, the PCR products were cloned into cloning vector pCR II (Invitrogen Corp.) for DNA sequencing.

LAMP assay of *aac(6′)-Iae*. The LAMP assay amplifies DNA with high specificity under isothermal conditions (31). To identify *P. aeruginosa* isolates possessing *aac(6′)-Iae*, we designed four primers (FIP, 5′-CAA TAC AAA TGT TTT CGG CGC TAC GTC ACT CCA AAA GGC TAC-3′; BIP, 5′-TAA ACG ATG AAT TGT GTG GTT GGG TTG GAT GTA GTT CCC AAG TT-3′; F3, 5′-TCA CAC ATA AAT TTC GAT TCT TG-3′; and B3, 5′-ACC AAA TCC CTT ATT TTG ATG TT-3′) for the LAMP assay. To extract DNA from *P. aeruginosa* isolates, a colony on *N*-acetyl-L-cysteine agar medium was suspended in 100 μl distilled water and boiled for 5 min. The bacterial suspension was then centrifuged at 12,000 × *g* for 2 min, and DNA in the supernatant was used for the LAMP assay. The LAMP reaction was performed with a Loopamp DNA amplification kit (Eiken Chemical Co., Ltd., Tokyo, Japan). The LAMP reaction mixture (12.5 μl), supplemented with 1.6 μM FIP and BIP primers, 0.2 μM F3 and B3 primers, 2× reaction mixture (6.25 μl), 4 U *Bst* DNA polymerase, 8 μg monomeric cyanine (YO-PRO-1), and 1.0 μl DNA sample, was incubated at 63°C for 45 min in a real-time thermal cycling system (Roter-Gene 2000; Corbett Research, Mortlake, New South Wales, Australia). Amplified DNA was monitored at 510 nm during the incubation. Alternatively, 25 μl of the reaction mixture was incubated at 63°C for 45 min on a block incubator (Advanced Science and Technology Enterprise Corp., Tokyo, Japan). After incubation, 10 μl of 1/100-diluted SYBR Green I nucleic acid gel stain (BioWhittaker Molecular Applications, Rockland, ME) was added to the reaction mixture. A change in color from orange to green indicated positive amplification.

PCR of QRDRs. The *gyrA*, *gyrB*, *parC*, and *parE* quinolone resistance-determining regions (QRDRs) were amplified by PCR with primers from and according to the methods described previously (1, 11, 20, 28). PCR products were sequenced with the same primers.

DNA sequencing. DNA sequences determined by the dideoxy chain termination method with an ABI PRISM 3100 sequencer (Applied Biosystems), and deduced protein sequences were subjected to homology searches in the DNA Data Bank of Japan (DDBJ), GenBank, and EMBL databases with FASTA and BLAST.

Pulsed-field gel electrophoresis (PFGE). Chromosomal DNA was prepared by the procedure of Grundmann et al. (10) and digested overnight with 10 U SpeI (Takara Bio, Inc., Shiga, Japan). The DNA fragments were separated on 1.0% agarose gels in 0.5× Tris-borate-EDTA buffer with a CHEF Mapper system (Bio-Rad Laboratories, Hercules, CA) at 6 V/cm for 20 h. The obtained fingerprinting patterns, normalized to the molecular weight markers, were analyzed by the unweighted-pair-group method with Molecular Analyst Fingerprinting Plus software, version 1.6 (Bio-Rad Laboratories, Inc.), to obtain average linkage-based dendrograms.

Statistical analysis. Results of a PCR assay, a LAMP assay, and an agglutination test were analyzed by chi-square test. A P value of <0.01 was considered statistically significant.

RESULTS

Distribution of MDR *P. aeruginosa* among hospitals. Nineteen hospitals and one clinical laboratory center from a single prefecture (population size, 2,360,000) participated in this study. MDR *P. aeruginosa* was isolated from 13 hospitals (Fig. 1). A total of 214 MDR *P. aeruginosa* isolates were obtained; 73 (34%), 38 (18%), and 22 (10%) were obtained from hospitals NA, CB, and CA, respectively, indicating that the spread of MDR *P. aeruginosa* was relatively limited. Seventy non-MDR *P. aeruginosa* isolates from the same hospitals were used for comparative analysis.

Serotyping. Ten serotypes were identified (Table 1): 222 were O11, 14 were O1, 10 were O10, 8 were B, 7 were M, 5 were O4, 4 were O3, 4 were O6, and 1 each was O9 and C. Six additional isolates showed agglutination with polyvalent antiserum but not with any of the monovalent antisera, i.e., they were nontypeable. A total of 212 of the 214 MDR *P. aeruginosa* isolates (99%) were serotype O11, whereas 70 of the non-MDR isolates were of a variety of serotypes, including O1, O3, O4, O6, O9, O10, O11, B, C, and M. These results indicated that serotype O11 was predominant for MDR *P. aeruginosa* in this prefecture.

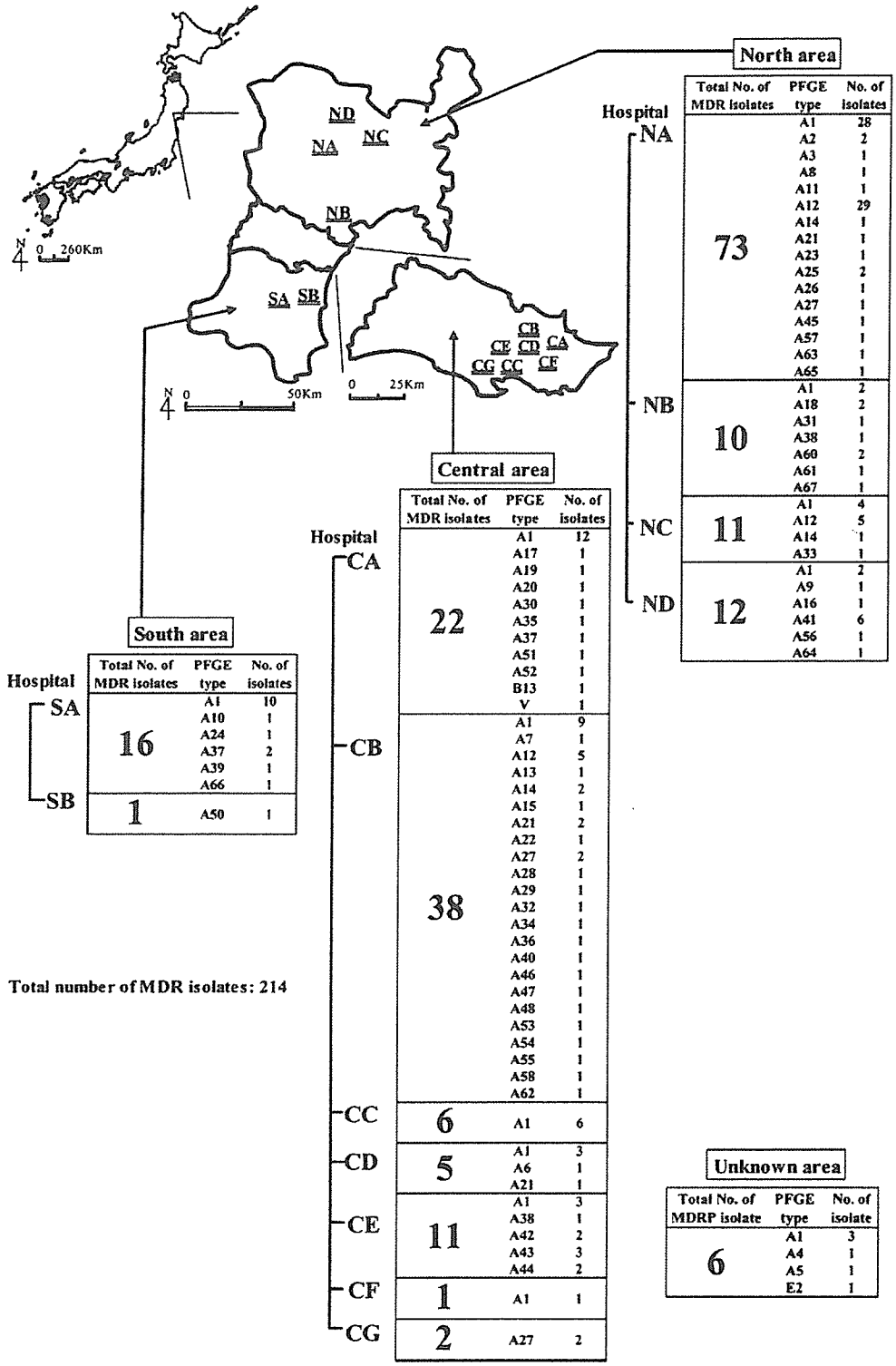

FIG. 1. Distribution of 214 isolates of MDR *P. aeruginosa* among 13 hospitals in Japan. Double capital letters indicate the locations of the hospitals that participated in this MDR *P. aeruginosa* survey.

Antimicrobial susceptibility tests. Most of the MDR *P. aeruginosa* isolates were resistant to all antimicrobials tested, except for GEN and PL-B (Tables 1 and 2). Rates of drug resistance were as follows: AMK, 100%; ABK, 91.6%; AZL, 99.5%; CAZ, 100%; CIP, 100%; DRPM, 99.1%; GEN, 57.5%; IPM, 100%; MEM, 100%; OFX, 100%; PIP, 100%; PL-B, 28%; STR, 100%; TZP, 100%. Rates of drug resistance among the non-MDR isolates were less than 63%, except that for

TABLE 1. Phenotypic and genotypic characterization of 284 clinical isolates of *P. aeruginosa*

No. of isolates	Susceptibility to:									Serotype	Gene cassette(s) of the class 1 integron	PFGE type(s)
	β-Lactams					Amino-glycosides		FQs[a]				
	PIP	TZP	CAZ	IPM	MEM	AMK	GEN	CIP	OFX			
MDR-*P. aeruginosa*[b]												
120	R	R	R	R	R	R	R	R	R	O11	bla_{IMP-1}, *aac(6′)-Iae*, *aadA1*	A1, A2, A4, A5, A7, A8, A9, A10, A12, A14, A15, A16, A18, A20, A21, A24, A25, A27, A28, A30, A31, A32, A33, A38, A41, A42, A43, A44, A45, A46, A48, A51, A54, A56, A62, A64, E2
85	R	R	R	R	R	R	S	R	R	O11	bla_{IMP-1}, *aac(6′)-Iae*, *aadA1*	A1, A2, A6, A11, A12, A13, A17, A18, A19, A21, A22, A23, A25, A26, A27, A34, A35, A36, A37, A39, A40, A41, A44, A47, A52, A53, A55, A58, A60, A61, A63, A65, A66, A67
1	R	R	R	R	R	R	R	R	R	O1	bla_{IMP-1}, *aac(6′)-Iae*, *aadA1*	A1
2	R	R	R	R	R	R	S	R	R	O1	bla_{IMP-1}, *aac(6′)-Iae*, *aadA1*	A38, A50
1	R	R	R	R	R	R	R	R	R	M	bla_{IMP-1}, *aac(6′)-Iae*, *aadA1*	A57
3	R	R	R	R	R	R	S	R	R	M	bla_{IMP-1}, *aac(6′)-Iae*, *aadA1*	A3, A29, A37
1	R	R	R	R	R	R	R	R	R	O10	*aac(6′)-31-like1*	B13
1	R	S	R	R	R	R	S	R	R	O1		V
Non-MDR-*P. aeruginosa*												
1	R	S	S	S	R	R	S	R	R	O11		A49
1	S	S	S	S	S	S	S	R	R	O11		A59
1	R	R	S	R	R	S	R	R	R	O1	*aac(6′)-31-like2*	B1
1	S	S	R	R	R	S	R	R	R	O1	*aac(6′)-31-like2*	B1
1	S	S	S	R	R	S	S	R	R	O1	*aac(6′)-31-like2*	B1
1	R	S	S	R	R	S	R	R	R	O1	*aac(6′)-31-like2*	B2
1	S	S	S	R	R	S	R	R	R	O1	*aac(6′)-31*	B6
1	S	S	S	R	R	S	R	R	R	O1	*aac(6′)-31-like1*	B8
1	R	S	S	R	R	S	S	R	R	O1	*aac(6′)-31-like1*	B7
1	S	S	S	S	S	S	S	R	R	O6	*aac(6′)-31-like1*	B3
1	S	S	S	S	S	S	S	R	R	O10	*aac(6′)-31-like1*	B4
1	S	S	S	R	R	S	R	R	R	O10	*aac(6′)-31-like1*	B5
1	S	S	S	R	R	S	S	R	R	O10	*aac(6′)-31-like1*	B9
1	S	S	S	R	S	S	R	R	R	O10	*aac(6′)-31*	B12
1	R	S	S	R	S	S	S	R	R	O10	*aac(6′)-31-like1*	B14
1	S	S	S	S	S	S	S	R	R	NT[c]	*aac(6′)-31*	B10
1	R	S	S	R	R	S	S	R	R	M	*aac(6′)-31-like1*	B11
2	R	R	R	R	R	S	S	R	R	NT		C1
1	R	R	R	R	R	S	S	R	R	O3		C2
2	R	R	R	R	R	S	S	R	R	O3		C4
1	S	S	S	R	R	S	S	R	R	O1		C3
1	S	S	R	R	R	S	S	R	R	O1		C7
1	R	R	R	R	R	S	S	R	R	B		C5
1	S	S	S	S	S	S	S	S	S	B		C6
1	R	R	R	R	R	S	S	R	R	O11		C8
1	S	S	S	S	S	S	S	S	R	O4		D1
1	S	S	S	S	S	S	S	R	R	O4		D2
1	S	S	S	S	S	S	S	R	S	O11		D3
1	S	S	S	R	R	S	S	R	R	O11		E1
1	R	S	S	R	R	S	S	R	R	M		F1
1	S	S	S	R	S	S	S	R	R	O4		F2
1	R	S	S	R	R	S	S	S	R	O11		G1
1	R	S	S	S	R	S	S	R	R	O11		G2
1	R	S	R	R	R	S	S	R	R	O11		H1
1	R	R	R	S	S	S	S	S	S	B		H2
2	S	S	S	R	R	S	S	S	S	O10		I
1	S	S	S	S	S	S	S	S	S	O4		J1
1	S	S	S	S	S	S	S	S	S	O3		J2
1	S	S	S	S	S	S	S	S	S	NT		K1
1	S	S	S	S	S	S	S	S	S	O6		K2
1	R	R	R	S	S	S	S	S	R	O9		L1
1	S	S	S	S	S	S	S	R	R	B		L2
1	R	S	S	S	S	S	R	R	R	O11	*aac(6′)-31-like3*, *aadA6*, *orfD*	M
1	R	R	R	R	R	S	R	R	R	B	bla_{IMP-1}, *aadA1*	N
1	R	S	S	S	S	S	S	S	S	O1		O
1	R	S	R	R	R	S	S	S	S	O6		P
1	S	S	S	S	S	S	S	S	S	C		Q
1	R	R	S	R	R	S	S	S	R	O10		R
1	S	S	S	S	S	S	S	S	S	O4		S

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TABLE 1—*Continued*

No. of isolates	Susceptibility to:									Serotype	Gene cassette(s) of the class 1 integron	PFGE type(s)
	β-Lactams					Amino-glycosides		FQs[a]				
	PIP	TZP	CAZ	IPM	MEM	AMK	GEN	CIP	OFX			
1	S	S	S	S	S	S	S	S	S	O11		T
1	S	S	S	S	S	S	S	S	S	O11		U
1	S	S	S	S	S	S	S	S	S	O11		W
1	S	S	S	S	S	S	S	S	S	O11		Z
1	S	S	S	S	S	S	S	S	S	O11		AA
1	S	S	S	S	S	S	S	S	S	O11		AJ
1	S	S	S	S	S	S	S	S	S	M		X
1	S	S	R	S	S	S	S	S	S	O1		Y
1	S	S	S	S	S	S	S	S	S	O10		AB
1	R	S	R	S	S	S	S	S	R	B		AC
1	S	S	S	S	S	S	S	S	S	O6		AD
1	R	R	R	S	S	R	S	S	S	O11		AE
1	S	S	S	S	S	R	R	S	S	O11		AF
1	R	R	S	S	S	S	S	S	S	NT		AG
1	R	S	S	S	S	S	S	S	S	B		AH
1	S	S	S	R	S	S	S	S	R	O1		AI
1	S	S	S	S	S	S	S	S	S	B		AK
1	S	S	S	S	S	S	S	S	S	NT		AL

[a] FQs, fluoroquinolones.
[b] Numbers of MDR isolates showing a respective PFGE type are shown in Fig. 1.
[c] NT, nontypeable.

STR, which was 98.6%. MIC_{50} and MIC_{90} values for MDR isolates were high, except those for ABK, GEN, and PL-B, and MIC_{50} and MIC_{90} values for non-MDR isolates were low, except those for AMK.

MBL production. MBL confers bacterial resistance to all β-lactams except AZL (53). Of the 284 isolates, 213 (75%) produced MBL and all except one were MDR isolates.

AAC(6′)-Iae production. AAC(6′)-Iae was first identified in MDR *P. aeruginosa* strain IMCJ2.S1 (46). We developed a slide agglutination test with AAC(6′)-Iae antibody-conjugated beads. *P. aeruginosa* IMCJ2.S1 showed a positive result within 30 s (Fig. 2, lane 2), whereas AAC(6′)-Iae-negative *P. aeruginosa* strain ATCC 27853 did not (Fig. 2, lane 4). Two hundred seventeen isolates were positive for the production of AAC(6′)-Iae in this test (Table 3). The results of the slide agglutination test were in complete agreement with Western blotting data obtained with AAC(6′)-Iae antibody (data not shown).

Detection of class 1 integrons. PCR assay with primers 5′-cs and 3′-cs (24), which are specific for the 5′ conserved segments (CS) (49) and the 3′ CS (49) of class 1 integrons, respectively, showed that 230 of the 284 isolates were positive. Amplified band sizes ranged from 0.8 kb to 2.5 kb (data not shown). All of these 230 isolates yielded a single band. Of these isolates, 212 yielded a 2.5-kb band, which is the same as that of the class 1 integron In113 (46). Sixteen isolates yielded a 0.8-kb band, and the remaining two yielded a 1.8-kb band and a 1.7-kb band. For the 212 isolates showing a 2.5-kb band, the presence of In113 was confirmed by PCR with specific primers, as described previously. MBL genes bla_{IMP-2} and bla_{VIM-2} are frequently found in Japan and are often associated with integrons (47). Therefore, we screened the 284 MDR *P. aeruginosa* iso-

TABLE 2. MIC_{50} and MIC_{90} values and percent antimicrobial resistance for 284 samples of *P. aeruginosa*

Antimicrobial agent	Breakpoint for resistance (μg/ml)	MDR isolates[a] (n = 214)				Non-MDR isolates (n = 70)			
		% Resistant	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Resistant	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
PIP	≥128	100	128–>512	>512	>512	41.4	1–>512	64	512
TZP	≥128/4	100	128–>512	512	>512	21.4	0.5–256	32	128
CAZ	≥32	100	32–>512	>512	>512	25.7	1–>512	8	64
IPM	≥16	100	32–>512	256	512	47.1	0.25–>512	8	32
DRPM	≥16	99.1	2–>512	>512	>512	34.3	<0.125–>512	8	32
MEM	≥16	100	32–>512	512	>512	44.3	<0.125–>512	4	32
AZT	≥32	99.5	16–>512	128	128	52.9	0.5–128	32	64
ABK	≥4	91.6	2–16	4	8	24.3	<0.125–16	1	8
AMK	≥32	100	32–256	128	256	2.9	0.25–256	2	16
GEN	≥16	57.5	0.25–>32	16	16	12.9	<0.125–>128	1	16
STR	≥4	100	512–>512	>512	>512	98.6	2–>512	32	128
CIP	≥4	100	16–>128	64	>128	51.4	<0.125–>128	4	64
OFX	≥8	100	32–>128	>128	>128	62.9	<0.125–>128	16	>128
PL-B	≥4	28.0	2–8	2	4	22.9	1–8	2	4

[a] Isolates defined as resistant to three antibiotics, imipenem (MIC ≥ 16 μg/ml), amikacin (MIC ≥ 32 μg/ml), and ciprofloxacin (MIC ≥ 4 μg/ml).

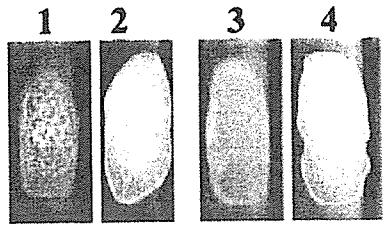

FIG. 2. Slide agglutination test with AAC(6′)-Iae antibody-conjugated beads. Lane 1, AAC(6′)-Iae positive control; lane 2, *P. aeruginosa* IMCJ2.S1 positive control; lane 3, 50 mM HEPES buffer negative control as solvent of AAC(6′)-Iae; lane 4, *P. aeruginosa* ATCC 27853 negative control.

lates for bla_{IMP-2} and bla_{VIM-2} by PCR. None of the 284 isolates were positive for bla_{IMP-2} or bla_{VIM-2}.

The regions between the 5′ CS and 3′ CS of amplicons of unexpected sizes were sequenced, and the gene cassettes were identified (Table 1). Of 16 isolates showing an 0.8-kb band, three possessed a single gene cassette containing *aac(6′)-31*, encoding 6′-*N*-aminoglycoside acetyltransferase type IV (R. E. Mendes, unpublished data; DDBJ/EMBL/GenBank accession no. AJ640197) (Table 1). This gene cassette was 639 nucleotides (nt) and contained a 65-nt 59-base-element (be) site, for site-specific cointegration events (35). Nine isolates possessed an *aac(6′)-31-like1* cassette identical to *aac(6′)-31*, with the exception of a C-to-T substitution at nt 269 in the coding region. Four isolates possessed an *aac(6′)-31-like2* cassette identical to *aac(6′)-31*, with the exception of a C-to-A substitution at nt 269. One isolate showing a 1.8-kb band possessed an array of three gene cassettes (Table 1). Of them, the first cassette was an *aac(6′)-31-like3* cassette similar to *aac(6′)-31* except for T-to-C and A-to-T substitutions at nt 57 and 266, respectively. The second cassette was 855 nt and contained the aminoglycoside adenylyltransferase gene *aadA6* (29) and a 60-nt 59-be site. The third cassette was 320 nt and contained open reading frame *orfD*, of unknown function (29). The *aadA6* and *orfD* cassettes were identical to those of In51 reported previously (29). One isolate showing a 1.7-kb band possessed two gene cassettes of bla_{IMP-1} (33) and *aadA1* (25) (Table 1).

Resistance to fluoroquinolones. Amino acid alterations to GyrA, GyrB, ParC, and ParE QRDRs of the 284 isolates are listed in Table 4. Amino acid replacement in the QRDR of GyrA (83Thr→Ile or 87Asp→Asn, Gly, or Tyr) was detected in 254 of the 284 isolates (89.4%). Of these 254 isolates, 8 possessed a mutation of GyrA alone. The remaining isolates possessed additional substitutions in GyrA, GyrB, ParC, and ParE. The 83Thr→Ile substitution in GyrA was the predominant replacement (251 of 284 isolates, 88.4%), in agreement with previous data on fluoroquinolone-resistant *P. aeruginosa* isolates (1, 22, 28). A double mutation of GyrA, 83Thr→Ile and 87Asp→Asn or Gly, was detected in nine isolates.

Amino acid replacement in the QRDR of ParC (87Ser→Leu or 91Glu→Lys) was detected in 244 of the 284 isolates (85.9%). All of these 244 isolates possessed additional mutations. The 87Ser→Leu substitution was the predominant replacement (242 of 284 isolates, 85.2%) and has been implicated in fluoroquinolone resistance of *P. aeruginosa* (1, 22, 28). A double mutation of ParC, 87Ser→Leu and 91Glu→Lys, was detected in three isolates. We found an 83Pro→Leu, 85Gly→Asp, and 88Ala→Pro alterations in one isolate each (Table 4).

Amino acid replacement in the QRDR of GyrB (468Glu→Asp) was detected in 70 of the 284 isolates (24.6%). No double mutations in GyrB were detected. Lee et al. (22) recently reported that 468Glu→Asp was a predominant alteration of GyrB, and isolates with this alteration, in addition to GyrA (83Thr→Ile) and ParC (87Ser→Leu) substitutions, showed a high level of resistance to CIP (MIC > 64 μg/ml). Our results were in accordance with their findings. We also found a 458Ala→Thr alteration in four isolates and a 496Ile→Val alteration in one isolate. These alterations are probably not associated with CIP resistance in *P. aeruginosa* because they were found in CIP-susceptible isolates.

Amino acid replacement in the QRDR of ParE (425Ala→Val or 459Glu→Asp or both) was detected in 30 of the 284 isolates (10.6%). All isolates possessed multiple mutations of ParE. Lee et al. (22) speculated that the 459Glu→Asp mutation of ParE is associated with moderate or high-level fluoroquinolone resistance in *P. aeruginosa*. The 425Ala→Val mutation has been reported in fluoroquinolone-resistant isolates of *P. aeruginosa* (1). Other mutations leading to amino acid changes were found at codons 419 (Asp→Asn, 1 isolate), 427 (Gln→Leu, 1 isolate), and 457 (Ser→Alg, 1 isolate). The fluoroquinolone

TABLE 3. Comparison of PCR, LAMP, and agglutination test results for the detection of MDR *P. aeruginosa* isolates belonging to genotype cluster A[a]

Isolates	No. of isolates with indicated result by:								
	PCR			LAMP			Agglutination test with AAC(6′)-Iae antibody-conjugated beads		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
MDR *P. aeruginosa*									
Cluster A	212	0	212	212	0	212	207	5	212
Other	0	2	2	0	2	2	0	2	2
Non-MDR *P. aeruginosa*									
Cluster A	0	2	2	0	2	2	0	2	2
Other	0	68	68	0	68	68	10	58	68
Total	212	72	284	212	72	284	217	65	284

[a] In all tests and combinations, the multidrug resistance of the isolates was positively associated with the positive results of *aac(6′)* tests based on chi-square tests ($P < 0.0001$).

TABLE 4. Amino acid changes in *gyrA*, *gyrB*, *parC*, and *parE* genes in 284 clinical isolates of *P. aeruginosa*

No. of strains (n = 284)	MIC (μg/ml) of:		Replacement in QRDRs[j]								
			GyrA at position:		ParC at position:			GyrB at position:		ParE at position:		
	CIP	OFX	83Thr (ACC)	87Asp (GAC)	87Ser (TCG)	91Glu (GAG)	Other	468Glu (GAG)	Other	425Ala (GCG)	459Glu (GAG)	Other
MDR *P. aeruginosa*												
1	>128	>128	Ile (ATC)	—[a]	Leu (TTG)	—	83Pro→Leu[b]	Asp (GAT)	—	—	Asp (GAT)	—
25	128–>128	>128	Ile (ATC)	—	Leu (TTG)	—	—	Asp (GAT)	—	—	Asp (GAT)	—
1	128	>128	Ile (ATC)	—	Leu (TTG)	—	—	Asp (GAT)	—	—	—	427Gln→Leu[g]
37	32–128	128–>128	Ile (ATC)	—	Leu (TTG)	—	—	Asp (GAT)	—	—	—	—
1	>128	>128	Ile (ATC)	Asn (AAC)	Leu (TTG)	Lys (AAG)	—	—	—	—	—	—
1	16	32	Ile (ATC)	—	Leu (TTG)	—	85Gly→Asp[c]	—	—	—	—	—
147	16–>128	32–>128	Ile (ATC)	—	Leu (TTG)	—	—	—	—	—	—	—
1	32	64	Ile (ATC)	—	—	—	—	—	—	—	—	457Ser→Arg[h]
Non-MDR *P. aeruginosa*												
5	64–>128	>128	Ile (ATC)	—	Leu (TTG)	—	—	Asp (GAT)	—	—	—	—
4	32–128	64–>128	Ile (ATC)	Asn (AAC)	Leu (TTG)	—	—	—	—	—	—	—
1	128	>128	Ile (ATC)	Asn (AAC)	Leu (TTG)	Lys (AAG)	—	—	—	—	—	—
1	>128	>128	Ile (ATC)	Asn (AAC)	—	Lys (AAG)	—	—	—	—	—	—
1	64	>128	Ile (ATC)	Asn (AAC)	Leu (TTG)	—	—	—	—	—	Asp (GAT)	—
1	64	128	Ile (ATC)	Gly (GGC)	Leu (TTG)	—	88Ala→Pro[d]	—	—	—	—	—
13	32–64	64–>128	Ile (ATC)	—	Leu (TTG)	—	—	—	—	—	—	—
2	16–32	32–128	Ile (ATC)	—	Leu (TTG)	—	—	—	—	Val (GTG)	—	—
1	16	128	Ile (ATC)	—	Leu (TTG)	—	—	—	458Ala→Thr[e]	—	—	—
1	16	128	Ile (ATC)	—	—	Lys (AAG)	—	—	—	—	—	—
1	16	128	Ile (ATC)	—	—	—	—	—	—	Val (GTG)	—	—
1	8	128	Ile (ATC)	—	—	—	—	—	458Ala→Thr	—	—	419Asp→Asn[i]
1	2	16	—	—	Leu (TTG)	—	—	—	458Ala→Thr	—	—	—
6	<0.25–0.5	1–8	—	—	—	—	—	—	458Ala→Thr	—	—	—
2	<0.25	0.25	—	—	—	—	—	—	496Ile→Val[f]	—	—	—
1	4	64	—	—	—	—	—	Asp (GAT)	—	—	—	—
5	0.5–4	8–16	Ile (ATC)	—	—	—	—	—	—	—	—	—
2	1–2	2–8	—	Tyr (TAC)	—	—	—	—	—	—	—	—
1	<0.25	0.25	—	—	—	—	—	—	—	—	—	—
20	<0.25–16	<0.25–64	—	Asn (AAC)	—	—	—	—	—	—	—	—

[a] —, no amino acid change.
[b] 83Pro→Leu, Pro at position 83 of ParC changed to Leu (CCG→CTG).
[c] 85Gly→Asp, Gly at position 85 of parC changed to Asp (GGC→GAC).
[d] 88Ala→Pro, Ala at position 88 of ParC changed to Pro (GCC→CCC).
[e] 458Ala→Thr, Ala at position 453 of GyrB changed to Thr (GCG→ACG).
[f] 496Ile→Val, Ile at position 496 of GyrB changed to Val (ATG→GTC).
[g] 427Gln→Leu, Gln at position 427 of ParE changed to Leu (CAG→CTG).
[h] 457Ser→Arg, Ser at position 457 of ParE changed to Arg (AGC→AGG).
[i] 419Asp→Asn, Asp at position 419 of ParE changed to Asn (GAC→AAC).
[j] Mutated nucleotides are underlined.

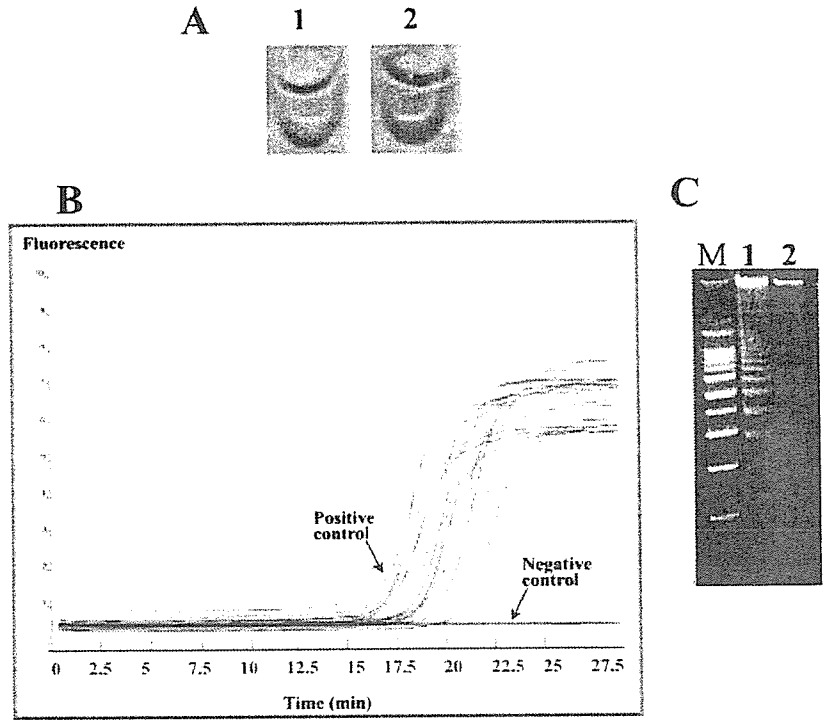

FIG. 3. LAMP assay to detect MDR *P. aeruginosa* isolates possessing the *aac(6')-Iae* gene encoding the aminoglycoside acetyltransferase AAC(6')-Iae. *P. aeruginosa* IMCJ2.S1 and ATCC 27853 were used as positive and negative controls, respectively. (A) Visual inspection analysis of LAMP products. Lane 1, *P. aeruginosa* IMCJ2.S1; lane 2, *P. aeruginosa* ATCC 27853. (B) Real-time amplification monitoring of *aac(6')-Iae*-specific LAMP. The amplification signal was detected at an average of 18 min, as indicated by the continuous increase in fluorescence. Increased fluorescence was not observed in the negative control. (C) Acrylamide gel electrophoresis of LAMP product. Lane 1, LAMP product of the 204-bp target sequence of the *aac(6')-Iae* gene of *P. aeruginosa* IMCJ2.S1; lane 2, *P. aeruginosa* ATCC 27853 negative control; lane M, 1-kbp ladder.

resistance associated with these mutations remains to be determined.

Analysis of the *aac(6')-Iae* gene by the LAMP method. To detect *aac(6')-Iae*, we developed a gene-specific LAMP assay. The index strain IMCJ2.S1 was used to standardize the method. Visual inspection showed that the LAMP assay successfully amplified the target sequence of the *aac(6')-Iae* gene of *P. aeruginosa* IMCJ2.S1 (Fig. 3A). Real-time kinetics of the LAMP reaction showed that the amplification signal could be detected on average by 18 min; fluorescence increased in the positive samples, following a sigmoid curve (Fig. 3B). Agarose gel electrophoresis of the LAMP products (Fig. 3C) showed a ladder-like pattern on the gel due to the formation of a mixture of stem-loop DNAs of various stem lengths, which are characteristic of LAMP products.

A total of 284 isolates, including 214 MDR *P. aeruginosa* isolates, were tested by the LAMP assay (Table 3). A total of 212 isolates were positive by the LAMP assay (Table 3). The results of the LAMP assay were in complete concordance with the PCR data, indicating that the PCR can be replaced by the LAMP method for detection of *aac(6')-Iae*-carrying *P. aeruginosa*. These results, together with ones of the agglutination test (Table 3), indicate that multidrug resistance was strongly associated with the presence of *aac(6')-Iae* and AAC(6')-Iae production in the *P. aeruginosa* isolates ($P < 0.0001$).

Genotyping by PFGE. The 284 isolates, including 214 MDR isolates, were typed by PFGE. One hundred thirty-three different PFGE types, designated from A1 to AL, were distinguished (Table 1). Fourteen types, A1, A2, A12, A14, A18, A21, A25, A27, A37, A41, A42, A43, A44, and A60, were identified in more than 2 isolates (Fig. 1), and type A1, which represented 83 of the isolates (29%), was the most prevalent and widely disseminated (Fig. 1), suggesting prefecture-wide clonal dissemination. Types A1, A12, A14, A21, A27, A37, and A38 were identified at two or more hospitals. Cluster analysis of the PFGE restriction patterns showed three large clusters, A, B, and C, sharing ≥70% similarity (Fig. 4). Of the 214 MDR isolates, 211 belonged to cluster A, comprising types A1 to A67, indicating that multidrug resistance was associated with one genotype, cluster A (Fig. 4 and Table 3). Fifteen isolates belonged to cluster B comprising types B1 to B14, and 10 isolates belonged to cluster C, comprising types C1 to C8. The PFGE patterns of the 35 non-MDR isolates varied greatly.

DISCUSSION

A clonal expansion of *P. aeruginosa* resistant to three antibiotics, carbapenems, amikacin, and fluoroquinolones, has been reported (4, 14, 36, 37, 46). However, previous surveillance studies in Japan have not shown clonal expansion involving multiple hospitals (19, 52). The present study showed clonal expansion of MDR *P. aeruginosa* in hospitals in the Tohoku area of Japan. To our knowledge, this is the first description of a large-scale, community-wide outbreak of nos-

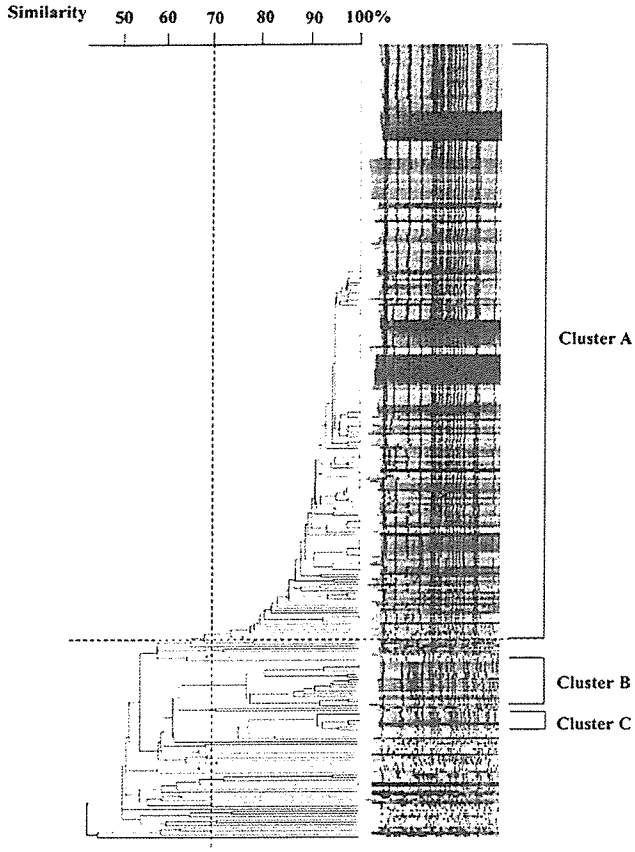

FIG. 4. Cluster analysis based on the PFGE patterns of 284 clinical isolates of *P. aeruginosa* from the 13 hospitals in the present study. Clustering was carried out with Molecular Analyst FingerprintingPlus software, version 1.6, as described in Materials and Methods.

ocomial infection caused by a single *P. aeruginosa* clone with high-level resistance to a large number of antibiotics. The routes of transmission of the MDR *P. aeruginosa* clone remain unclear. *P. aeruginosa* that can be recovered from the hospital environment could be a possible source of nosocomial infection (6, 42, 54). Patient-to-patient transmission has been documented among patients with cystic fibrosis (5, 42, 54). Catheter-associated urinary tract infections appeared widespread among the hospitals in our study; the majority of the isolates (approximately 70%) were obtained from urine specimens, and approximately 80% of these were from patients with urinary catheters.

Most MDR isolates tested (205 of 214; Table 1) showed a serotype of O11. This was not surprising because these isolates belonged to a single cluster, as revealed by PFGE analysis (Fig. 4). *P. aeruginosa* is categorized into 31 chemotypes, including 20 IATS serotypes and subtypes (48). Thus far, however, particular serotypes, such as serotypes O12 and O11, appear to have been preferentially associated with *P. aeruginosa* outbreaks (9, 23, 38, 41). A clone of *P. aeruginosa* belonging to serogroup O12, which was resistant to both carbenicillin and gentamicin, was predominant in outbreaks involving six hospitals in Athens in 1987 (23). Later, O12 isolates resistant to these two drugs were reported in European countries (9, 38, 41). *P. aeruginosa* O12 resistant to ciprofloxacin and ceftazidime and/or fosfomycin was implicated in hospital outbreaks in France during the period 1993 to 1994 (3). *P. aeruginosa* serotype O11 caused hospital outbreaks in the 1980s in the United States (8) and in 1994 and 1995 in Greece (50). *P. aeruginosa* O11 was implicated in folliculitis caused by the use of whirlpools and hot tubs in the 1970s and 1980s in the United States and Canada (40). More recently, hospital outbreaks caused by MDR *P. aeruginosa* serotype O11 occurred in Belgium (5) and in Japan (46). Different strains of serotype O11 were involved in the above-mentioned outbreaks because their PFGE profiles were quite different. In addition, the Japanese strains produced IMP-1 carbapenemase (46), but the Belgian strains did not (5). It is not known why *P. aeruginosa* strains belonging to particular serotypes of O12 and O11 were involved in these outbreaks.

We analyzed several features including serotype, antimicrobial susceptibility, MBL production, prevalence of *aac(6')-Iae*, structure of class 1 integrons, resistance to fluoroquinolones, and genotype based on PFGE analysis for MDR *P. aeruginosa* isolates. Results indicated that *aac(6')-Iae* is a good candidate marker for MDR *P. aeruginosa* infection. To detect the *aac(6')-Iae* gene and its product, we developed a LAMP-based detection assay and an agglutination assay. LAMP is a nucleic acid amplification method which relies on autocycling strand displacement DNA synthesis performed by the *Bst* DNA polymerase large fragment (31). The amplification products are stem-loop DNA structures with several inverted repeats of the target and cauliflower-like structures with multiple loops. LAMP assays are simple and short and do not require expensive equipment. LAMP assays have been applied to the analysis of various infectious agents such as hepatitis B virus (7), *Mycobacterium tuberculosis* (15), severe acute respiratory syndrome coronavirus (13), *E. coli* O157:H7 (27), *Clostridium difficile* (18), *Bordetella pertussis* (17), *Salmonella enterica* (32), *Mycoplasma pneumoniae* (43), and *Streptococcus pneumoniae* (45). The LAMP assay developed in this study was as sensitive and specific as PCR. Though less sensitive and specific than the LAMP assay, the agglutination assay for AAC(6')-Iae is sufficiently accurate to detect MDR *P. aeruginosa* (98% of MDR *P. aeruginosa* isolates were positive). The agglutination assay is simpler and cheaper than the LAMP assay and is also useful in detecting MDR *P. aeruginosa* in the clinical setting.

MDR *P. aeruginosa* may have spread across Japan as a result of the increasing use of carbapenems such as IPM, aminoglycosides such as AMK, and fluoroquinolones such as CIP. Nationwide surveillance for MDR *P. aeruginosa* is under way. At the hospital level, monitoring for environmental sources of bacteria, cleaning of contaminated surfaces of treatment rooms and bathrooms, review of infection control measures in the treatment of urine, and avoidance of unnecessary measurements of urine are considered effective in preventing *P. aeruginosa* nosocomial infections. Although the mode of transmission between hospitals is unknown, the movement of infected patients from one hospital to another is a possibility. Thirty-one patients infected with MDR *P. aeruginosa* had been transferred from other hospitals to the hospitals participating in the present study.

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REFERENCES

1. **Akasaka, T., M. Tanaka, A. Yamaguchi, and K. Sato.** 2001. Type II topoisomerase mutations in fluoroquinolone-resistant clinical strains of *Pseudomonas aeruginosa* isolated in 1998 and 1999: role of target enzyme in mechanism of fluoroquinolone resistance. Antimicrob. Agents Chemother. **45:** 2263–2268.
2. **Arakawa, Y., N. Shibata, K. Shibayama, H. Kurokawa, T. Yagi, H. Fujiwara, and M. Goto.** 2000. Convenient test for screening metallo-beta-lactamase-producing gram-negative bacteria by using thiol compounds. J. Clin. Microbiol. **38:**40–43.
3. **Bingen, E., S. Bonacorsi, P. Rohrlich, M. Duval, S. Lhopital, N. Brahimi, E. Vilmer, and R. V. Goering.** 1996. Molecular epidemiology provides evidence of genotypic heterogeneity of multidrug-resistant *Pseudomonas aeruginosa* serotype O:12 outbreak isolates from a pediatric hospital. J. Clin. Microbiol. **34:**3226–3229.
4. **Clinical and Laboratory Standards Institute.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th ed. Approved standard M07-A7. Clinical and Laboratory Standards Institute, Wayne, PA.
5. **Deplano, A., O. Denis, L. Poirel, D. Hocquet, C. Nonhoff, B. Byl, P. Nordmann, J. L. Vincent, and M. J. Struelens.** 2005. Molecular characterization of an epidemic clone of panantibiotic-resistant *Pseudomonas aeruginosa*. J. Clin. Microbiol. **43:**1198–1204.
6. **Doring, G., S. Jansen, H. Noll, H. Grupp, F. Frank, K. Botzenhart, K. Magdorf, and U. Wahn.** 1996. Distribution and transmission of *Pseudomonas aeruginosa* and *Burkholderia cepacia* in a hospital ward. Pediatr. Pulmonol. **21:**90–100.
7. **Enomoto, Y., T. Yoshikawa, M. Ihira, S. Akimoto, F. Miyake, C. Usui, S. Suga, K. Suzuki, T. Kawana, Y. Nishiyama, and Y. Asano.** 2005. Rapid diagnosis of herpes simplex virus infection by a loop-mediated isothermal amplification method. J. Clin. Microbiol. **43:**951–955.
8. **Farmer, J. J., III, R. A. Weinstein, C. H. Zierdt, and C. D. Brokopp.** 1982. Hospital outbreaks caused by *Pseudomonas aeruginosa*: importance of serogroup O11. J. Clin. Microbiol. **16:**266–270.
9. **Grattard, F., O. G. Gaudin, B. Pozzetto, A. Ros, and A. D. Mbida.** 1993. Genotypic homogeneity of nosocomial *Pseudomonas aeruginosa* O12 strains demonstrated by analysis of protein profiles, DNA fingerprints and rRNA gene restriction patterns. Eur. J. Clin. Microbiol. Infect. Dis. **12:**57–61.
10. **Grundmann, H., C. Schneider, D. Hartung, F. D. Daschner, and T. L. Pitt.** 1995. Discriminatory power of three DNA-based typing techniques for *Pseudomonas aeruginosa*. J. Clin. Microbiol. **33:**528–534.
11. **Hocquet, D., X. Bertrand, T. Kohler, D. Talon, and P. Plesiat.** 2003. Genetic and phenotypic variations of a resistant *Pseudomonas aeruginosa* epidemic clone. Antimicrob. Agents Chemother. **47:**1887–1894.
12. **Homma, J. Y.** 1982. Designation of the thirteen O-group antigens of *Pseudomonas aeruginosa*: an amendment for the tentative proposal in 1976. Jpn. J. Exp. Med. **52:**317–320.
13. **Hong, T. C., Q. L. Mai, D. V. Cuong, M. Parida, H. Minekawa, T. Notomi, F. Hasebe, and K. Morita.** 2004. Development and evaluation of a novel loop-mediated isothermal amplification method for rapid detection of severe acute respiratory syndrome coronavirus. J. Clin. Microbiol. **42:**1956–1961.
14. **Hsueh, P. R., L. J. Teng, P. C. Yang, Y. C. Chen, S. W. Ho, and K. T. Luh.** 1998. Persistence of a multidrug-resistant *Pseudomonas aeruginosa* clone in an intensive care burn unit. J. Clin. Microbiol. **36:**1347–1351.
15. **Iwamoto, T., T. Sonobe, and K. Hayashi.** 2003. Loop-mediated isothermal amplification for direct detection of *Mycobacterium tuberculosis* complex, *M. avium*, and *M. intracellulare* in sputum samples. J. Clin. Microbiol. **41:**2616–2622.
16. **Jones, R. N., T. R. Anderegg, J. M. Swenson, and The Quality Control Working Group.** 2005. Quality control guidelines for testing gram-negative control strains with polymyxin B and colistin (polymyxin E) by standardized methods. J. Clin. Microbiol. **43:**925–927.
17. **Kamachi, K., H. Toyoizumi-Ajisaka, K. Toda, S. C. Soeung, S. Sarath, Y. Nareth, Y. Horiuchi, K. Kojima, M. Takahashi, and Y. Arakawa.** 2006. Development and evaluation of a loop-mediated isothermal amplification method for rapid diagnosis of *Bordetella pertussis* infection. J. Clin. Microbiol. **44:**1899–1902.
18. **Kato, H., T. Yokoyama, and Y. Arakawa.** 2005. Rapid and simple method for detecting the toxin B gene of *Clostridium difficile* in stool specimens by loop-mediated isothermal amplification. J. Clin. Microbiol. **43:**6108–6112.
19. **Kimura, S., J. Alba, K. Shiroto, R. Sano, Y. Niki, S. Maesaki, K. Akizawa, M. Kaku, Y. Watanuki, Y. Ishii, and K. Yamaguchi.** 2005. Clonal diversity of metallo-β-lactamase-possessing *Pseudomonas aeruginosa* in geographically diverse regions of Japan. J. Clin. Microbiol. **43:**458–461.
20. **Kureishi, A., J. M. Diver, B. Beckthold, T. Schollaardt, and L. E. Bryan.** 1994. Cloning and nucleotide sequence of *Pseudomonas aeruginosa* DNA gyrase *gyrA* gene from strain PAO1 and quinolone-resistant clinical isolates. Antimicrob. Agents Chemother. **38:**1944–1952.
21. **Laraki, N., M. Galleni, I. Thamm, M. L. Riccio, G. Amicosante, J. M. Frere, and G. M. Rossolini.** 1999. Structure of In31, a bla_{IMP}-containing *Pseudomonas aeruginosa* integron phyletically related to In5, which carries an unusual array of gene cassettes. Antimicrob. Agents Chemother. **43:**890–901.
22. **Lee, J. K., Y. S. Lee, Y. K. Park, and B. S. Kim.** 2005. Alterations in the GyrA and GyrB subunits of topoisomerase II and the ParC and ParE subunits of topoisomerase IV in ciprofloxacin-resistant clinical isolates of *Pseudomonas aeruginosa*. Int. J. Antimicrob. Agents **25:**290–295.
23. **Legakis, N. J., N. Koukoubanis, K. Malliara, D. Michalitsianos, and J. Papavassiliou.** 1987. Importance of carbenicillin and gentamicin cross-resistant serotype 0:12 *Pseudomonas aeruginosa* in six Athens hospitals. Eur. J. Clin. Microbiol. **6:**300–303.
24. **Levesque, C., L. Piche, C. Larose, and P. H. Roy.** 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob. Agents Chemother. **39:**185–191.
25. **Liebert, C. A., R. M. Hall, and A. O. Summers.** 1999. Transposon Tn*21*, flagship of the floating genome. Microbiol. Mol. Biol. Rev. **63:**507–522.
26. **Liu, P. V., H. Matsumoto, H. Kusama, and T. Bergan.** 1983. Survey of heat-stable, major somatic antigens of *Pseudomonas aeruginosa*. Int. J. Syst. Bacteriol. **33:**256–264.
27. **Maruyama, F., T. Kenzaka, N. Yamaguchi, K. Tani, and M. Nasu.** 2003. Detection of bacteria carrying the *stx2* gene by in situ loop-mediated isothermal amplification. Appl. Environ. Microbiol. **69:**5023–5028.
28. **Mouneimne, H., J. Robert, V. Jarlier, and E. Cambau.** 1999. Type II topoisomerase mutations in ciprofloxacin-resistant strains of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **43:**62–66.
29. **Naas, T., L. Poirel, and P. Nordmann.** 1999. Molecular characterisation of In51, a class 1 integron containing a novel aminoglycoside adenylyltransferase gene cassette, aadA6, in *Pseudomonas aeruginosa*. Biochim. Biophys. Acta **1489:**445–451.
30. **Nakamura, A., M. Hosoda, T. Kato, Y. Yamada, M. Itoh, K. Kanazawa, and H. Nouda.** 2000. Combined effects of meropenem and aminoglycosides on *Pseudomonas aeruginosa* in vitro. J. Antimicrob. Chemother. **46:**901–904.
31. **Notomi, T., H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino, and T. Hase.** 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. **28:**E63.
32. **Ohtsuka, K., K. Yanagawa, K. Takatori, and Y. Hara-Kudo.** 2005. Detection of *Salmonella enterica* in naturally contaminated liquid eggs by loop-mediated isothermal amplification, and characterization of *Salmonella* isolates. Appl. Environ. Microbiol. **71:**6730–6735.
33. **Osano, E., Y. Arakawa, R. Wacharotayankun, M. Ohta, T. Horii, H. Ito, F. Yoshimura, and N. Kato.** 1994. Molecular characterization of an enterobacterial metallo-β-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. Antimicrob. Agents Chemother. **38:**71–78.
34. **Pagani, L., C. Colinon, R. Migliavacca, M. Labonia, J. D. Docquier, E. Nucleo, M. Spalla, M. Li Bergoli, and G. M. Rossolini.** 2005. Nosocomial outbreak caused by multidrug-resistant *Pseudomonas aeruginosa* producing IMP-13 metallo-beta-lactamase. J. Clin. Microbiol. **43:**3824–3828.
35. **Partridge, S. R., G. D. Recchia, C. Scaramuzzi, C. M. Collis, H. W. Stokes, and R. M. Hall.** 2000. Definition of the attI1 site of class 1 integrons. Microbiology **146:**2855–2864.
36. **Pellegrino, F. L. P. C., L. M. Teixeira, M. da Glória Siqueira Carvalho, S. Aranha Nouer, M. Pinto De Oliveira, J. L. Mello Sampaio, A. D'Ávila Freitas, A. L. P. Ferreira, E. de Lourdes Teixeira Amorim, L. W. Riley, and B. M. Moreira.** 2002. Occurrence of a multidrug-resistant *Pseudomonas aeruginosa* clone in different hospitals in Rio de Janeiro, Brazil. J. Clin. Microbiol. **40:**2420–2424.
37. **Pirnay, J. P., D. De Vos, C. Cochez, F. Bilocq, J. Pirson, M. Struelens, L. Duinslaeger, P. Cornelis, M. Zizi, and A. Vanderkelen.** 2003. Molecular epidemiology of *Pseudomonas aeruginosa* colonization in a burn unit: persistence of a multidrug-resistant clone and a silver sulfadiazine-resistant clone. J. Clin. Microbiol. **41:**1192–1202.
38. **Pitt, T. L., D. M. Livermore, D. Pitcher, A. C. Vatopoulos, and N. J. Legakis.** 1989. Multiresistant serotype O 12 *Pseudomonas aeruginosa*: evidence for a common strain in Europe. Epidemiol. Infect. **103:**565–576.
39. **Pournaras, S., M. Maniati, E. Petinaki, L. S. Tzouvelekis, A. Tsakris, N. J. Legakis, and A. N. Maniatis.** 2003. Hospital outbreak of multiple clones of

Pseudomonas aeruginosa carrying the unrelated metallo-beta-lactamase gene variants bla_{VIM-2} and bla_{VIM-4}. J. Antimicrob. Chemother. **51**:1409–1414.
40. **Ratnam, S., K. Hogan, S. B. March, and R. W. Butler.** 1986. Whirlpool-associated folliculitis caused by *Pseudomonas aeruginosa*: report of an outbreak and review. J. Clin. Microbiol. **23**:655–659.
41. **Richard, P., R. Le Floch, C. Chamoux, M. Pannier, E. Espaze, and H. Richet.** 1994. *Pseudomonas aeruginosa* outbreak in a burn unit: role of antimicrobials in the emergence of multiply resistant strains. J. Infect. Dis. **170**:377–383.
42. **Saiman, L., and J. Siegel.** 2004. Infection control in cystic fibrosis. Clin. Microbiol. Rev. **17**:57–71.
43. **Saito, R., Y. Misawa, K. Moriya, K. Koike, K. Ubukata, and N. Okamura.** 2005. Development and evaluation of a loop-mediated isothermal amplification assay for rapid detection of *Mycoplasma pneumoniae*. J. Med. Microbiol. **54**:1037–1041.
44. **Sambrook, J., and D. W. Russell.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, New York, NY.
45. **Seki, M., Y. Yamashita, H. Torigoe, H. Tsuda, S. Sato, and M. Maeno.** 2005. Loop-mediated isothermal amplification method targeting the *lytA* gene for detection of *Streptococcus pneumoniae*. J. Clin. Microbiol. **43**:1581–1586.
46. **Sekiguchi, J., T. Asagi, T. Miyoshi-Akiyama, T. Fujino, I. Kobayashi, K. Morita, Y. Kikuchi, T. Kuratsuji, and T. Kirikae.** 2005. Multidrug-resistant *Pseudomonas aeruginosa* strain that caused an outbreak in a neurosurgery ward and its *aac(6')-Iae* gene cassette encoding a novel aminoglycoside acetyltransferase. Antimicrob. Agents Chemother. **49**:3734–3742.
47. **Shibata, N., Y. Doi, K. Yamane, T. Yagi, H. Kurokawa, K. Shibayama, H. Kato, K. Kai, and Y. Arakawa.** 2003. PCR typing of genetic determinants for metallo-β-lactamases and integrases carried by gram-negative bacteria isolated in Japan, with focus on the class 3 integron. J. Clin. Microbiol. **41**: 5407–5413.
48. **Stanislavsky, E. S., and J. S. Lam.** 1997. *Pseudomonas aeruginosa* antigens as potential vaccines. FEMS Microbiol. Rev. **21**:243–277.
49. **Stokes, H. W., and R. M. Hall.** 1989. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol. Microbiol. **3**:1669–1683.
50. **Tassios, P. T., V. Gennimata, A. N. Maniatis, C. Fock, N. J. Legakis, and The Greek *Pseudomonas aeruginosa* Study Group.** 1998. Emergence of multidrug resistance in ubiquitous and dominant *Pseudomonas aeruginosa* serogroup O:11. J. Clin. Microbiol. **36**:897–901.
51. **Tsuchizaki, N., K. Ishino, F. Saito, J. Ishikawa, M. Nakajima, and K. Hotta.** 2006. Trends of arbekacin-resistant MRSA strains in Japanese hospitals (1979 to 2000). J. Antibiot. **59**:229–233.
52. **Tsuji, A., I. Kobayashi, T. Oguri, M. Inoue, E. Yabuuchi, and S. Goto.** 2005. An epidemiological study of the susceptibility and frequency of multiple-drug-resistant strains of *Pseudomonas aeruginosa* isolated at medical institutes nationwide in Japan. J. Infect. Chemother. **11**:64–70.
53. **Walsh, T. R., M. A. Toleman, L. Poirel, and P. Nordmann.** 2005. Metallo-beta-lactamases: the quiet before the storm? Clin. Microbiol. Rev. **18**:306–325.
54. **Zimakoff, J., N. Hoiby, K. Rosendal, and J. P. Guilbert.** 1983. Epidemiology of *Pseudomonas aeruginosa* infection and the role of contamination of the environment in a cystic fibrosis clinic. J. Hosp. Infect. **4**:31–40.

Development of information retrieval and web information integration system for nosocomial infection anecdotal research papers

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Abstract—**Sharing infectious information is very effective to correspond and prevent infection outbreak. In past, we have developed a pilot nosocomial infection anecdotal research database system on the web to access easily. This system is able to search target research papers using some categories, but are only able to list papers with abstract. Therefore, If we wanted to survey the a focused infection, we must read all retrieved documents and choose target data like number of patients or isolate numbers. In this article, we would like to suggest using natural language processing technique and extracting numeric information which is in documents in order to integrate web data. Consequently, we are able to develop web information integrated system which can extract numeric data in which retrieved research papers crossly.**

Index Terms—**nosocomial infection anecdotal research papers, web data integration system, numeric information.**

I. Introduction

SHERING Infectious information is very effective when we adapt outbreak situation. For instance, we can know what infection disease is outbreak now around them, and how they corresponded the infection disease in past. Therefore we can settle this outbreak fast and adequately. In past, we have developed a pilot nosocomial infection anecdotal research database system to access easily [1][2]. This system contained more than 350 infection outbreak research papers with some categorize factors, which are date, hospital, pathogen, journal, study methods and so on, and we opened this system to the public on World Wide Web as "web-based database". So, user was able to highly structured abstracts. These categories which we decided as important factor for nosocomial infection research papers are very useful because we became possible focusing and retrieving these papers according to our demand. However, we are not able to survey in order to grasp crossover and keyword search, if we used this system. For instance, most of patients number is in body of a paper, and we are not able to treat the number as "data" which are calculated and analyzed usually. Therefore, we considered that we were able to make information integration system[3] for infection outbreak database, if we used selectable categories and numeric values.

In this article, we would like to suggest using natural language processing technique and extracting numeric information which is in documents in order to integrate web data and showing new retrieve information system.

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II. Method

A. Target

Our target database is "Hospital Infection Outbreak Database" website [4], and this site has database in which this system has 362 nosocomial infection anecdotal research papers. Most papers dealt with epidemiological investigations. A few outbreaks due to non-infectious origin were included, such as acute onset of diminished vision and hearing in dialysis patients, pseudo-outbreaks[2]. A webpage was created for the web-based database search. The website presents study background, instructions for use and search menu in English and Japanese. The search menu has category search interface and a user is able to select from a pull down menu of choices, which are pathogens, infection sites, modes of transmission, types of investigation and word/service. These infection articles naturally have some numeric values, which are various numbers, values, grade and so on, for example, total number of patient, number of relevant health care workers etc.

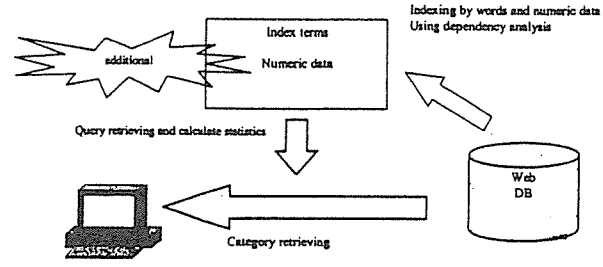

Fig.1. system concept

B. Information integration technology and numeric information

Information integration technology [3] has been focus of constant attention of web information beneficial use. If we are able to treat piecemeal data of web as integrated data, Value of web data was enhanced very much. In general, the information integration is three main processes [5]:

1. Collecting web pages where necessary information is described.
2. Extracting relevant information from the web pages
3. Relating the relevant information

Recently World Wide Web information integrated system is used agent technology. All web pages are not able to be processed by simple program because web data structure are very various. So, the agent technology is adopted in order to effect mutually.

Numeric information is very important in not only web pages but also any other documents. However, it is difficult that we treat numeric value correctly if the numeric values are in free text documents. When we want to extract useful information from free text data, we are able to use various natural language processing techniques. In particular, we considered that a dependency structure analysis [6] is useful when we would extract numeric value and relevant words, because dependency information informed relations of certain numeric value and corresponding word. Dependency structure analysis consists of two steps. In the first step, dependency matrix is constructed, in which each element corresponds to a pair of chunks and represents the probability of a dependency relation between them. The second step is to find the optimal combination of dependencies to form entire sentences.

C. *Tool developing*

We adapt a dependency analysis in order to extract numeric values and words to which the numeric values related. We developed extracting tool of numeric values and corresponding words. This tool was able to extract numeric information in free text documents. Next, we developed information retrieval system which targeted papers using query in order to narrow the search to user's wants and could do crossover survey based on numeric information. This system wrapped to the pilot nosocomial infection anecdotal research database system and added these new functions. For example, user could do keyword search on this database and this system could display some statistics (e.g. mean of patients numbers) of retrieved papers. This tool implemented on the *"Hospital Infection Outbreak Database"* website. Figure 1 shows concept of this tool.

III. RESULT

The tools of dependency analysis extracted numeric values and corresponding words. number of numeric information in these reports was 2987 in these infection outbreak research papers. We could calculate numeric data in which we had deal with papers.

Table 1 shows one of instance of statistical data as follows;

Numeric information was appended each infection research papers and we could extract and calculate basic statistics. This tool gives all infection research papers its numeric data when it does indexing process.

Next, we implemented this function of dependency analysis and retrieving using keywords as integration information retrieval system is able to display search output with statistics. Users had been able to retrieve his and hers demanding information using keywords and get numeric statistics of these data. Of course, this system implemented on prior system as adding in. Figure 2, 3 show interfaces of this system. Section 1 is query field adding this time, section 2 is the prior system in figure 2. Section 3 in figure 3 is some statistics of numeric information (number of patients, week, Health care Workers (HCWs)).

TABLE I
STATISTIC OF NUMERIC VALUES AND CORRESPONDING WORDS

correspondig words	numbers	Mean	S.D.	Median
patients	366	33.364	79.433	10
days	174	15.293	22.464	9
cases	119	17.485	28.057	8
months	93	7.461	7.555	6
weeks	61	6.109	7.275	3
isolates	50	173.568	810.207	15
years	49	12.637	19.875	5
hours	35	102.531	207.594	48
strains	32	15.032	17.142	6
infants	30	47.793	127.376	9
HCWs	28	57.321	69.633	26.5

S.D.: Standard deviation

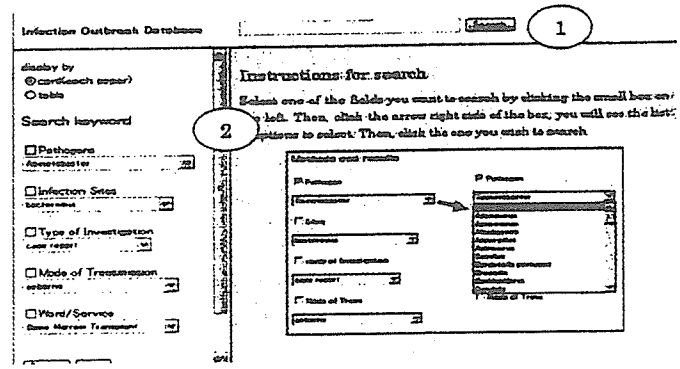

Fig.2. Interface of retrieval system (previous search)

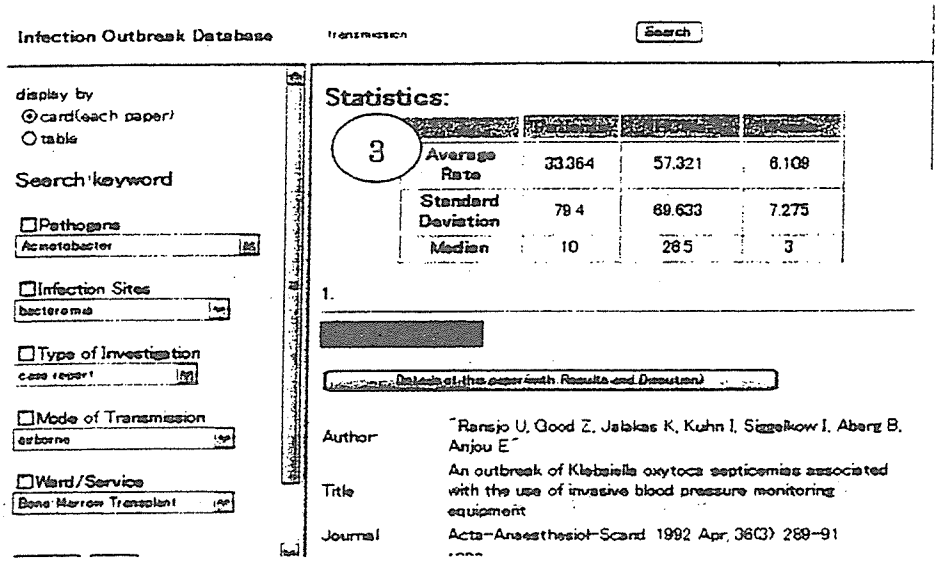

Figure3. Interface of retrieval system (result)

In this paper, we tried to extract relation between numeric data and corresponding words from the viewpoint of information integration. Concretely, we made the tool of dependence structure analysis and extracting relation numeric value and corresponding words. In addition, we made keyword search interface on prior epidemiological web database present essential information. We became possible the calculation of basic statistic in each retrieval result and we can expect radius of impact as numeric data if a similar outbreak is found using category and keyword.

However, all relation of extracted numeric data and corresponding words is not always correct when dependence analysis or syntax analysis, because all numeric data is not expression of typical numeric data. For example, an abstract of one research paper have three infants data if the infants which infect divide two category from a point of view and the research paper refer to these infants. In these cases, it is very difficult to distinct tellingly which numeric data is typical and dependent and we must use more advanced technique as semantic analysis. Currently semantic analysis had Almost all typical corresponding words (patients, cases, weeks and so on), however, are referred one times in each abstracts because this nosocomial outbreak investigation database collects typical cases.

Consequently, we developed web information integration system and were able to survey using the numeric information and display basic statistics in retrieved nosocomial infection anecdotal research papers.

[1] N.Ashida, T.Takemura, K.Makimoto, T.Kirikae. S.Suto, A Development of the Nationwide Report-gathering Network System to Prevent Nosocomial Infection. Healthcom2005, pp101-105, 2005.
[2] K.Makimoto, N. Ashida, N. Qureshi, T.Tsuchida, A.Skikawa, Development of a Nosocomial Outbreak Investigation database, Journal of Hospital Infection, 59, pp215-219, 2005
[3] M.A.Hearst, Information integration, IEEE intelligent systems, vol13, No.5, pp12-24, 1998
[4] The hospital infection outbreak homepage, http://www.health-db.net/infection/index.asp (last see : Jun 29, 2006)
[5]]H.Kohno, et.al. Information retrieval and agent. Tokyo denki diagaku press, 2002 (in Japanse)
[6] Taku kudo, Yuji Matsumoto, Japanese Dependency Analysis Based on Support Vector Machines, EMNLP/VLC 2000

studied a dementia's wandering patterns in a nursing home, using an automatic monitoring system with IC tags. Observation studies on wanderings action are conducted in three nursing homes in Japan and South Korea. This system which was intended to automatically record wondering observation results also recorded dementia elders' routine actions. Therefore, we can also use this system as an automatic reporting system for residents' families. Method: Several trigger fields were set up in a nursing home and nursing home staff and the residents wore IC tags. When an IC tag is in a trigger field, it transmits its ID and the trigger field ID, which tells the information about "Who", When" and "Where". The information is automatically recorded and classified according to ADL. A summarized daily profile is sent to a family of a resident by e-mail. Results: We developed two systems, one is to automatically record daily profiles by using IC tag system and the other is to send them by e-mail. Discussion: This automatic monitoring system through the use of IC tags improves the communication between a nursing home and residents' families, providing information on a daily basis for families without increasing the burden on nursing home staff.

References:

1. Ashida N., Makimoto K, Noraki C., Nakagawa M., Suzuki M. and Kawahara T.: A Monitoring of Wandering Behavioral Pattern of Senile Dementia with IC Tag..3rd APT TELEMEDICINE Workshop 2005. Proceedings p80-84.2005
2. Ashida N., Ashida Y., Takagishi Y. and Kawahara T.: The Survivor Search System using an IC Tag.4th APT Telemedicine Workshop 2006 p220-224.Pakistan 25-26. Jan 2006.

Web-Based Information Services for the Nationwide Model Project Against Hospital Infection

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Introduction: The Internet has been increasing its importance as an information collection tool. Nevertheless, reliable and good web sites tend to contain a massive amount of information and have complicated structures, which provides poor accessibility of desired information. We promoted the nationwide model project against hospital infection with the aims of 1) establishing the system for medical institutions so that they can consult specialists on a daily basis about hospital infection prevention through the specialized consultation service desks which are set up in different regions, and 2) connecting these regional desks with the International Medical Center of Japan with intent to transfer consulted cases online to the center so that the cases can be gathered to construct the system which is designed to provide information about remedies and safeguards. In Japan, it is difficult to have hospital infection cases reported, but, many research papers about hospital infection are written overseas. We extracted and summarized these anecdotal reports to create a database for Web-based information provision. This study is to report such Internet-based information gathering and provision for hospital infection prevention.

Method: The following three systems were constructed to gather and provide information about hospital infection prevention on the Web. 1. Nosocomial infection report system to gather consultation cases nationwide 2. Database of summaries of papers on nosocomial infection 3. Reliable search engine which specializes in hospital infection

Result: The nosocomial infection report system of Item 1 is operated only among registered institutions. The systems of Item 2 and 3 which are designed for web applications are experimentally operated at the following address. http://www.health-dh.net/infection/index.asp Moreover, the text analysis was performed on the contents which contained particular sentences. Coded data acquired from such contents were used for searches and the cross-disciplinary survey.

Discussion: Many manuals have been developed based on the CDC guideline in recognition of importance of preventive measures against hospital infection as a part of medical risk control. But, it is pointed out that the majority of them are like schoolbooks and require further decision to be made in the field without providing adequate instructions. It is also pointed out that they do not always provide a clear "definition of infection" which immediately becomes necessary in developing safeguards and remedies to "do what in which case." Prevention of hospital infection inevitably requires not only manuals but also the knowledge-based applications in selecting a solution based on cases. Gathering information from many web sites is also necessary in addition to gathering case examples, however, it frequently takes time to verify the reliability of information (if it is an official announcement, knowledge, or a personal view) when such information is obtained on the web site which is hit by a versatile search engine. Therefore, the development of a reliable, specialized search engine is desired.

Development of a Numeric Data Extracting and Analysis System Using Dependency Analysis on Nosocomial Outbreak Investigation Database

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Background: Sharing infectious information is very effective to correspond and prevent infection outbreak[1]. For instance, we can know what infection disease is outbreak now around them, and how they corresponded the infection disease in past. Therefore we can settle this outbreak fast and adequately. Therefore, to inform this kind of infection disease, nosocomial infection anecdotal research paper summaries database was developed [2]. This database has more than 350 summarized records of outbreak case reports and anyone uses the database on World Wide Web. This database has few categories and main body. These are specific categories which show each nosocomial infection anecdote, for instance, author, journal, pathogen, case definition and so on. These categories are very useful to retrieve his target document efficiently, but are only listing paper list with abstract. Therefore, If we wanted to survey the a focused infection, we must read all retrieved documents and choose target data like patient or isolate numbers. On this time, we developed that numeric information extracting system using modification structure[3] in order to treat web data based on free text as database[4], and survey system based on result of choosing infectious information. Method: We used 362 nosocomial infection anecdotal research papers, which were collected to develop the database system mentioned above. These infection papers did dependency analysis by which numeric information revealed relation of other words. Next, we developed information retrieval system which targeted these papers using query in order to narrow the search to user's wants and could do crossover survey based on numeric information. Result: number of numeric data in these reports was 2987. Some examples of numeric data are about patient, health care worker (HCW), things of infection and so on. For example, Some Specific data are "patients: 366 occurrences", "days: 174 occurrences", "cases: 119 occurrences", "isolates: 50 occurrences", "infants: 30 occurrences", "HCWs: 28 occurrences" in all 362 papers. So, this result leaded to calculate crossover statistic. For instance, it was possible to calculate basic statistics of "patients" in these papers (mean = 33, Standard Deviation = 79.44, median = 10). When a user narrowed the search using the retrieval system, it was able to calculate these statistical indexes depending on the search results. Conclusion: In this paper, we tried to extract relation between numeric data and dependency words with dependency analysis. We became possible the calculation of basic statistic in each retrieval result. Consequently, we were able to survey using the numeric data and do diversified analysis in nosocomial infection anecdotal research papers.

References:

1. N.Ashida, T.Takemura, K.Makimoto, T.Kirikae, S.Suto, A Development of the Nationwide Report-gathering Network System to Prevent Nosocomial Infection, Healthcom2005, pp101-105, 2005
2. K.Makimoto, N. Ashida, N. Qureshi, T.Tsuchida, A.Skikawa, Development of a Nosocomial Outbreak Investigation database, Journal of Hospital Infection, 59, p215-219, 2005
3. Taku kudo, Yuji Matsumoto, Japanese Dependency Analysis Based on Support Vector Machines, EMNLP/VLC 2000
4. S.Chawathe, et. al. The TSIMMIS project: Integration of Heterogeneous Information Sources, Proceedings of IPSJ Conference, pp.7-18, 1994