症例 1

血液培養から高度耐性腸内細菌科細菌が検出された 1 例

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（症例）76 歳　男性
（基礎疾患）十二指腸乳頭部癌　亜全胃温存膵頭十二指腸切除術術後
（既往歴）閉塞性胆管炎

（現病歴）X 年 5 月に上腹部痛と発熱が出現．症状はその後改善したが，MRI/CT で胆嚢結石・総胆管結石・主膵管の拡張を認め 7 月に当院紹介受診．ERCP を施行したところ，十二指腸乳頭部に腫瘤を指摘された．ERBD チューブ留置後一旦退院（処置に伴う予防化学療法として 1 日 CPZ/SBT を使用）．同部位の生検にて，adenocarcinoma と診断したため，十二指腸乳頭部癌・胆嚢結石・肝内結石・総胆管結石に対して亜全胃温存膵頭十二指腸切除術施行目的で X 年 9 月に再度入院となった．

9 月 10 日手術が実施され，術後に膵液漏や，それに伴う総肝動脈の仮性動脈瘤を認めた．しばしばドレーン入れ替えを行い，ドレナージを継続し，各種抗菌薬投与が行われた．改善と増悪を繰り返したのち，10 月 20 日膵上下部に留置されたドレーンの排液および 10 月 22 日の血液培養より多剤耐性の *Escherichia coli* が検出され，感染症内科にコンサルトとなった．Table 1 には *E. coli* の感受性結果，Fig. 1 に感染症内科コンサルトまでの経過と，Fig. 2 に使用抗菌薬および菌検出状況を示す．尚，同定感受性試験に関しては NMIC/ID-33（BD®）のコンボパネルを用い，全自動感受性測定試験システム（BD phoenix®）で行い，M100-S19 CLSI ブレイクポイントを基準として用いた．

（経過）ドレーンおよび血液から検出された *E. coli* は，カルバペネムを除く β-ラクタム薬に耐性を認めており，追加検査として clavulanate acid/amoxicillin disc（BD®）を用いたダブルディスクシナジーテスト，sodium mercaptoacetate disk（栄研化学®）を用いた SMA 法を行った．その結果，同定された *E. coli* は Extended spectrum β-lactamase（ESBL）ならびに metallo-β-lactamase（MBL）を産生していると考えられた．

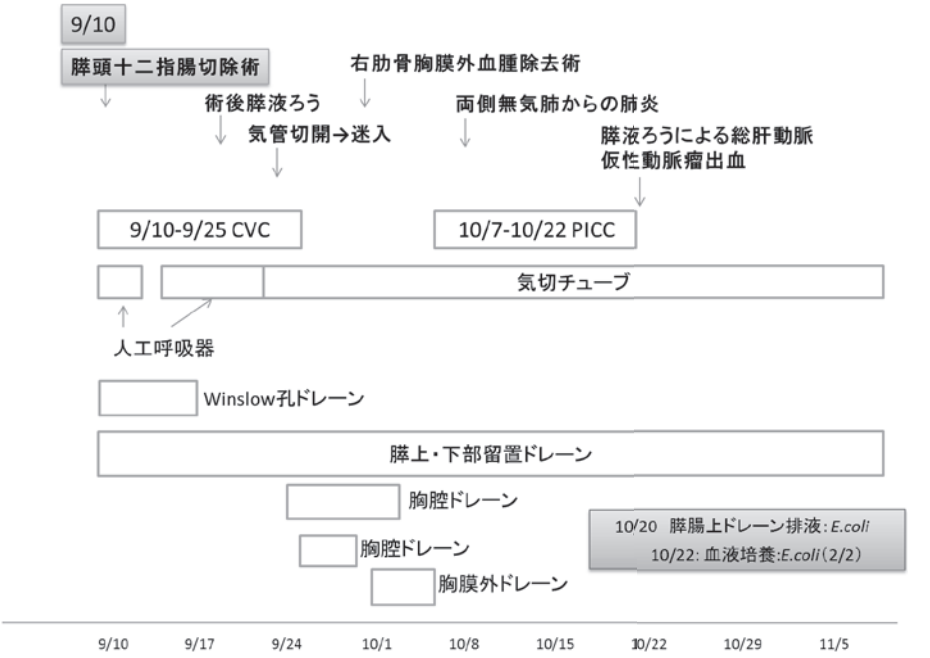
抗菌薬は，minimum inhibiratory concentration（MIC）値を参考に，meropenem と amikacin を選択した．投与量および投与方法は，meropenem 2g を 3 時間かけて投与し，amikacin 15mg/kg を腎機能に応じて投与調節して治療を行った．その結果，発熱は軽快し，ドレーン排液からは *Stenotrophomonas maltophilia* を少量認めるのみとなり，2 週間で抗菌薬治療は終了とした．その後，腹腔内ドレーンの入れ替えを行いながら，腹腔内膿瘍は改善と増悪を繰り返したのち，ドレーンを抜去することができ，順調に術後のリハビリを続けていた．

Table 1　10/22 に検出された *E. coli* の感受性

薬剤名	MIC（μg/mL）	感受性報告
ABPC	＞＝32	R
PIPC	＞＝128	R
CEZ	＞＝32	R
CAZ	＞＝64	R
CMZ	＞＝64	R
CFPM	＞＝32	R
CPZ/SBT	＞＝64	R
AZT	＞＝32	R
MEPM	＜＝1	
IPM/CS	＜＝1	
AMK	＝8	S
MINO	＝8	I
LVFX	＞＝8	R
CPFX	＞＝8	R

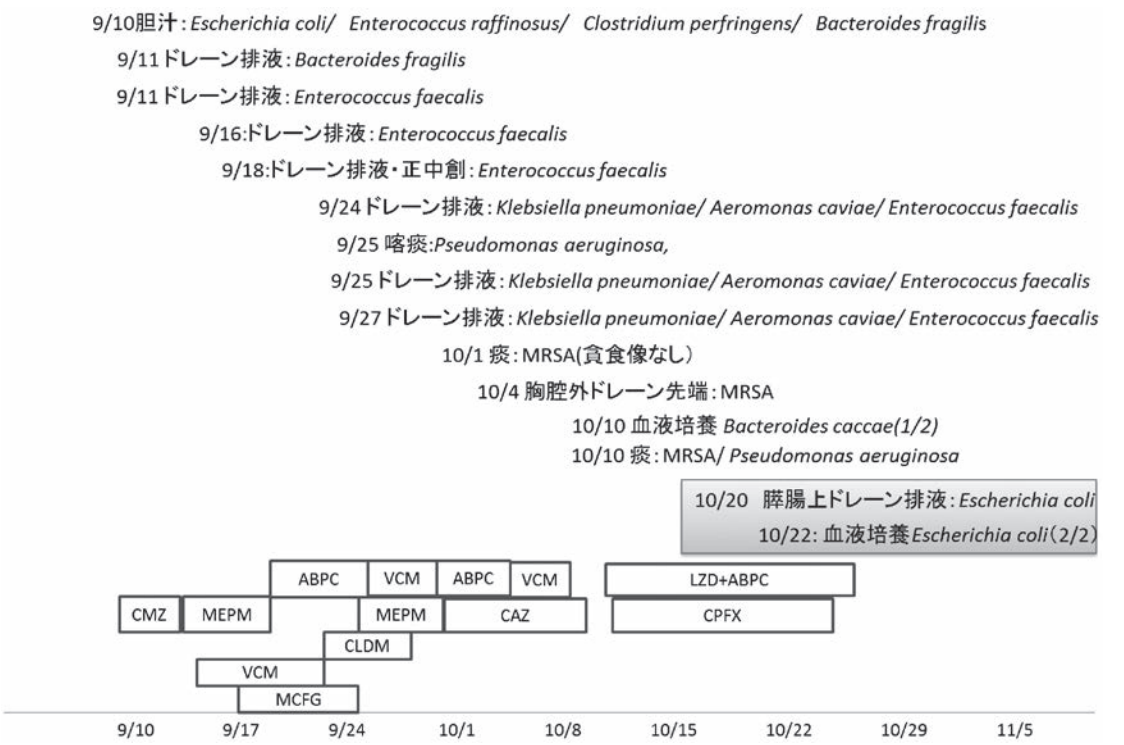
ABPC：ampicillin，PIPC：piperacillin，CEZ：cefamezine，CAZ：ceftazidime，CMZ：cefmetazole，CFPM：cefepime，CPZ/SBT：cefoperazone/sulbactam，AZT：aztreonam，MEPM：meropenem，IPM/CS：imipenem/cilastatin，AMK：amikacin，MINO：minocycline，LVFX：levofloxacin，CPFX：ciprofloxacin

Fig. 1　多剤耐性の *E. coli* が検出されるまでの臨床経過



CVC：central venous catheter，PICC：peripherally inserted central catheter

Fig. 2　多剤耐性の *E. coli* が検出されるまでの菌同定状況および治療歴



ABPC：ampicillin，CAZ：ceftazidime，CLDM：clindamycin，CMZ：cefmetazole，CPFX：ciprofloxacin，LZD：linezolid，MCFG：micafungin，MEPM：meropenem，VCM：vancomycin

しかし，X＋1年3月に肝膿瘍を指摘され，その際のドレナージ排液から，再度MBL産生の *E. coli* ならびに *Klebsiella oxytoca* とMBL非産生の *Pseudomonas aeruginosa*，*Bacteroides fragilis* が分離同定された（Table 2）．ドレナージを行いつつ，膿瘍への移行性を考え，meropenem 2g　3時間かけての

Table 2　肝膿瘍穿刺液の培養・感受性結果

	E. coli（MBLs）		*K. oxytoca*（MBLs）		*P. aeruginosa*		*Bacteroides fragilis*	
	MIC（μg/mL）		MIC（μg/mL）		MIC（μg/mL）		MIC（μg/mL）	
ABPC	＞＝32	R	＞＝32	R			＞＝2	R
ABPC/SBT	＞＝32	R	＞＝32				＝1	S
IPM/CS	＜＝1		＜＝1				＝1	S
MEPM	＜＝1		＝16	R	＝4	S	＝1	S
CLDM							＞＝8	R
MINO	＝8	I	＝8	I	＞＝16	R	＝1	S
PIPC	＞＝128	R	＞＝128	R	＜＝4	S		
AMK	＜＝4	S	＜＝4	S	＜＝8	S		
CFPM	＞＝32	R	＝8	R	＝16	I		
AZT	＝16	R	＝16	R	＝4	S		
LVFX	＞＝8	R	＞＝8	R	＞＝8	R		
CPFX	＞＝8	R	＝4	R	＞＝4	R		

MBLs：metallo-β-lactamases，*E.coli*：*Escherichia coli*，*K. oxytoca*：*Klebsiella oxytoca*，*P. aeruginosa*：*Pseudomonas aeruginosa*，ABPC：ampicillin，PIPC：piperacillin，CEZ：cefamezine，CAZ：ceftazidime，CMZ：cefmetazole，CFPM：cefepime，CPZ/SBT：sefoperazone/sulbactam，AZT：aztreonam，MEPM：meropenem，IPM/CS：imipenem/cilastatin，AMK：amikacin，MINO：minocycline，LVFX：levofloxacin，CPFX：ciprofloxacin

投与に加えtigecycline併用による点滴治療を行った．その結果，ドレーン排液の性状も改善し，解熱したが，投与8日目に掻痒感を伴う，融合傾向のある全身性皮疹と40℃までの発熱が出現した．tigecyclineによる薬剤性過敏症候群と考え，tigecyclineをamikacinに変更し，加療を継続したところ，軽快した．肝膿瘍は，治療開始から4週間の治療を行い，軽快退院となっている．尚，その後の検査で，今回同定された腸内細菌科細菌のMBLは，IMP型であることが判明している．

（診断）MBL産生腸内細菌科細菌（IMP型）による術後腹腔内膿瘍および肝膿瘍

（症例の疑問点から研究的考察へ）

本症例はIMP型の耐性遺伝子を獲得した腸内細菌科細菌による感染症である．薬剤感受性パターンから，MBLを初めとするβ-lactamase遺伝子の存在を考え，追加検査を施行したところ，ESBLならびにMBL産生菌であることが判明した．現在，カルバペネム耐性腸内細菌科細菌に対する治療に関しては，確立されたものはない．更に，海外では違う遺伝子型（VIM/KPC型など）をもつ腸内細菌科細菌感染症に関しては報告があるものの，今回検出されたIMP型でのまとまった報告はない．本症例は他の遺伝子型でのカルバペネム耐性腸内細菌科細菌の治療戦略として用いられる高用量・長時間投与のカルバペネム投与（2g/回，3時間以上かけての点滴静注）に加え[1)~3)]，感受性の残っていたアミノグリコシドを併用し，軽快に至った．

今後，本邦では同様の症例が増加していく可能性は高いと考えられるため，下記の4つの疑問点をあげ議論することとした．

本症例（MBL産生菌による感染症）の疑問点

1．MBL産生菌の疫学に関して

2．MBL産生の腸内細菌はなぜESBLも伴っていたのか？

3．MBL産生菌が他の腸内細菌科細菌でも認められたのはどういう機序によるものか？逆に*P. aeruginosa*がMBL産生遺伝子を獲得していなかった理由はあるのか？

謝辞　今回の症例に関して遺伝子解析を行って頂いた国立感染症研究所細菌第二部の鈴木里和先生，松井真理先生に謝辞を申し上げます．

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“本症例の疑問点”から“研究的考察”へ

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本症例（MBL 産生菌による感染症）の疑問点

1. MBL 産生菌の疫学に関して
2. MBL 産生の腸内細菌はなぜ ESBL も伴っていたのか？
3. MBL 産生菌が他の腸内細菌科細菌でも認められたのはどういう機序によるものか？逆に緑膿菌が MBL 産生遺伝子を獲得していなかった理由はあるのか？

はじめに

β-ラクタム系薬は，その安全性，殺菌性から，臨床の現場で最も頻繁に使用され，臨床医が最も使い慣れている有用な抗菌薬である．イミペネム，メロペネム，パニペネムなどのカルバペネム系薬は，グラム陰性桿菌の外膜透過性が良好で強力な殺菌力を主体とした抗菌作用を示すことから，感染症治療において重用されている．したがって，このカルバペネム系薬を良好な基質として加水分解することができるメタロ-β-ラクタマーゼは，感染症治療において脅威となる酵素である．

1. MBL 産生菌の疫学

β-ラクタマーゼは，β-ラクタム系薬共通の母核であるβ-ラクタム環を開環させる加水分解酵素の総称で，1980 年に Ambler はβ-ラクタマーゼのアミノ酸一次配列（DNA 塩基配列）を基に，クラス A～D 型の 4 クラスに分類した[1]．このクラス分類では，セリンを活性部位とするクラス A 型，C 型，D 型酵素をセリン-β-ラクタマーゼ，活性保持に亜鉛を必要とするクラス B 型酵素をメタロ-β-ラクタマーゼと称している．メタロ-β-ラクタマーゼは，活性中心に 1～2 分子の亜鉛を保持していて，EDTA などのキレート剤によって失活し，亜鉛などの 2 価の金属イオンを添加するとある程度の活性が回復する．

メタロ-β-ラクタマーゼとして，IMP 型や VIM 型及び SPM 型などの酵素が知られている[2]．また近年，New-Delhi metallo-β-lactamase-1（NDM-1）など新規メタロ-β-ラクタマーゼが発見され[3]，本邦でも数例の報告例がみられ話題となっている．海外で検出されるメタロ-β-ラクタマーゼは VIM-2 が優位であるが，本邦では海外と異なりほとんどが IMP-1 であり，IMP-2 及び VIM-1，VIM-2 の検出は稀である[4)5)]．

本邦で優位となっている IMP 型酵素産生遺伝子の多くはプラスミド上に存在する[6]．このプラスミドは伝達性で，1991 年に Watanabe らが本邦で初めて発見し，耐性遺伝子が伝達性プラスミドにより媒介されている緑膿菌の検出を報告している[7]．現在 IMP 型酵素はその亜型として 51 の型があり（平成 27 年 4 月 20 日現在），その多くが本邦で発見されたものである．

先述のように，これまで本邦で分離されるメタロ-β-ラクタマーゼは IMP 型が優位であり，そのほとんどは IMP-1 であった．ところが近年，肺炎桿菌，大腸菌を中心に IMP-6 産生菌の増加が報告されて始めている[8)9)]．

IMP-6 は 2001 年に我々のグループが *Serratia marcescens* から検出し報告したものである[10]．IMP-6 は，IMP-1 の構造遺伝子で 640 番目のアデニンがグアニンに変異しており，196 番目のアミノ酸がセリンからグリシンに置換したものである．このアミノ酸置換により，カルバペネム系薬に対する基質特異性が変化しており，IMP-1 はメロペネムよりイミペネムをより効率よく分解する酵素であるが，IMP-6 はイミペネムよりメロペネムをより分解することのできる酵素である．

我々が本邦で検出された IMP-6 型酵素を 2001 年に報告して以来，本酵素の流行，拡散の報告はしばらくみられなかったが，2012 年，本邦で肺炎桿菌から IMP-6 産生菌が 5 株分離されたことを Shigemoto らが報告している[8]．また，我々も 2012 年に本邦全域から収集した大腸菌のなかで SMA test 陽性 54 株について解析したところ，IMP-6 が 49 株，IMP-1 が 5 株であり，IMP-6 の分離率が非常に高い状況にある[9]．

2. MBL 産生の腸内細菌はなぜ ESBL も伴っていたのか？

先述した Ambler のクラス分類では，それぞれのクラスの酵素は，基質特異性に基づく分類にも対応

でき，クラスA型はペニシリナーゼ，クラスC型はセファロスポリナーゼ，クラスD型はオキサシリン分解型ペニシリナーゼ，クラスB型はカルバペネマーゼとなる．メタロ-β-ラクタマーゼは，カルバペネム系薬を良好な基質として加水分解する酵素であるが，カルバペネム系薬以外にもペニシリン系薬やセフェム系薬を分解することができ，他のクラスのβ-ラクタマーゼに比べて基質特異性の広い酵素である．また，メタロ-β-ラクタマーゼはクラスA型β-ラクタマーゼとは異なり，クラブラン酸やスルバクタムなどのβ-ラクタマーゼ阻害薬によって阻害されないが[11]，モノバクタム系薬の分解は苦手としている．

これまでIMP-6を含めたメタロ-β-ラクタマーゼの多くは病原性が低いとされる緑膿菌やアシネトバクターなどブドウ糖非発酵グラム陰性桿菌からの分離が主に報告されていた．しかし，近年の本邦におけるIMP-6産生株は，本症例でもみられたように比較的病原性の高い肺炎桿菌や大腸菌から検出されていることが注目されている．また，本邦から報告されたIMP-6産生肺炎桿菌，および大腸菌の多くはCTX-M-2を同時に産生していることが知られている．すなわち，染色体性にAmpCを産生しない肺炎桿菌や産生量の非常に少ない大腸菌であっても，メタロ-β-ラクタマーゼが分解を苦手とするモノバクタム系薬はCTX-M型酵素により分解されてしまうため，IMP-6産生株の治療にβ-ラクタム系薬が使用できないという問題点が生じる．本症例においてMBL産生菌がなぜESBLを保有しているのかは明らかではないが，おそらく，ESBLを同時産生することにより，MBLが苦手としているモノバクタム系薬をも分解できることになり，すなわち，より基質特異性を広くすることで，抗菌薬によるプレッシャーのもと生存していく可能性を高めているものと推測される．

3. MBL産生菌が他の腸内細菌科細菌でも認められたのはどういう機序によるものか？逆に緑膿菌がMBL産生遺伝子を獲得していなかった理由はあるのか？

プラスミドの型別として，Incompatibility（不和合性群）が用いられる[12]．腸内細菌科のプラスミドは27種類のInc groupsが知られている．Inc遺伝子はreplicationをコントロールする遺伝子に関連し，同じreplication systemを持つプラスミド同士はシステムを競合してしまうため，同じIncのプラスミドは同一菌体内に共存することができない．IMP-6を産生するプラスミドの多くはIncNと報告されている[8]．また，本症例のプラスミドもIncNであったことが国立感染症研究所により解析されている．Incの重要な点は，Inc groupsによりプラスミドがhostとする菌種や，プラスミドの伝達頻度に特徴がみられることにある．すなわち，Inc groupsを決定することにより，今後，どのような菌種にプラスミドが拡がっていくのかの動向予測につなげることが出来ることにある．IMP-6を産生するIncNプラスミドは，*E. coli*，*Klebsiella* spp.をhostとすることが多く，非発酵菌をhostとしにくいことが知られている．今回の症例で，他の腸内細菌科細菌で認められ，緑膿菌に認められなかったのは，プラスミドがIncNであったためと推測される．また，今後も*E. coli*，*Klebsiella* spp.を中心にIMP-6産生菌が分離されていくことが予測される．

おわりに

IMP-6産生菌の別の問題点として，IMP-6型酵素産生肺炎桿菌および大腸菌のイミペネムのMICが1μg/mL以下となることが多いという点が挙げられる．通常のルーチン薬剤感受性検査においては，カルバペネム系薬の代表検査薬としてイミペネムが使用されることが一般的であり，IMP-6産生株の多くがCLSIのスクリーニング基準で感性（S）に判定される．そのため，IMP-6産生株がメタロ-β-ラクタマーゼ産生菌であると臨床医に認知されない場合がある．Weisenbergらは，カルバペネム系薬を分解する酵素KPCを産生するKPC産生肺炎桿菌感染症において，薬剤感受性試験で感性（S）と判定された患者にカルバペネム系薬を使用した場合，予後不良となることを報告している[13]．IMP-6もKPCと同様にカルバペネム系薬を加水分解する酵素であることから，IMP-6産生菌にカルバペネム系薬を使用するとKPC産生菌の場合と同様のことが発生することが懸念される．今後，これらIMP-6産生株が病院検査室レベルで正しく検出される検査方法の確立が急務と考えられる．

文献

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J Infect Chemother (2013) 19:956–958
DOI 10.1007/s10156-012-0520-6

CASE REPORT

Three cases of IMP-type metallo-β-lactamase-producing *Enterobacter cloacae* bloodstream infection in Japan

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Received: 6 September 2012 / Accepted: 6 November 2012 / Published online: 19 November 2012

Abstract We report three cases of IMP-type metallo-β-lactamase-producing *Enterobacter cloacae* bloodstream infection, which showed minimum inhibitory concentration values for imipenem with 2 μg/ml in all isolates. Although carbapenems were initiated empirically in all cases, two of three cases died. The Clinical and Laboratory Standards Institute lowered the breakpoints of carbapenems for *Enterobacteriaceae* in 2010. However, the previous breakpoints are still used in many clinical laboratories, which can result in failure to detect carbapenem-resistant *Enterobacteriaceae*. Therefore, lower breakpoints of carbapenems should be used in clinical settings, and alternative tests for detecting metallo-β-lactamase such as polymerase chain reaction and immunochromatographic assays may contribute to better detection of carbapenem-resistant isolates.

Keywords Metallo-β-lactamase · Carbapenemase · *Enterobacter cloacae* · Bloodstream infection

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Introduction

Since metallo-β-lactamase (MBL)-producing *Enterobacter cloacae* was first reported in 2000, there have been increasing reports of carbapenem-resistant *E. cloacae* worldwide [1, 2]. MBL-producing *Enterobacteriaceae*, including *E. cloacae*, often exhibited relatively low minimum inhibitory concentrations (MIC) of carbapenems (0.06–2 μg/ml) [3]. Recently, the Clinical and Laboratory Standards Institute (CLSI) lowered the breakpoints of carbapenems against *Enterobacteriaceae* [4]. However, higher MIC breakpoints are still used in many clinical laboratories because Food and Drug Administration (FDA)-approved breakpoints have not been changed, which can result in failure to detect carbapenem-resistant *Enterobacteriaceae* [5]. Furthermore, it is unclear whether carbapenems are effective against MBL-producing *E. cloacae* [2]. Clinical studies to determine appropriate chemotherapeutic regimens against MBL-producing *E. cloacae* infection are required. Here, we report three cases including two with unsuccessful outcomes of IMP-type MBL-producing *E. cloacae* bloodstream infections in a hospital in Japan.

Case reports

Case 1 was a 91-year-old man who was admitted to the hospital for cerebral infraction. He developed aspiration pneumonia on day 20 and was treated with ampicillin/sulbactam (ABPC/SBT) for 10 days. He developed septic shock consequent to peripheral venous catheter infection on day 33, and administration of meropenem (MEM) was initiated. However, hemodynamic instability persisted, and blood culture revealed *E. cloacae* with elevated MIC for imipenem (IPM) (MIC = 2 μg/ml) and *Proteus vulgaris*. MEM was changed to levofloxacin (LVFX) based on the results of susceptibility testing on day 37. Furthermore, MBL production was tested using Cica-β-test [6] and an immunochromatographic assay [7] because of the elevated MIC for imipenem, which revealed positive. IMP-1 was confirmed by polymerase chain reaction and sequencing in our research institute. Although an additional blood culture was negative for *E. cloacae*, the patient died on day 40.

Case 2 was a 77-year-old man with type 2 diabetes receiving insulin therapy. He was admitted to the hospital for esophageal cancer, and subtotal esophageal resection and subcutaneous reconstruction were performed. He developed infection in the cervical wound because of leakage and received vancomycin and MEM for 36 days, with subsequent oral LVFX for 1.5 months. Although the surgical wound infection improved, he had recurrent aspiration pneumonia. On postoperative day (POD) 105, he developed bacteremia caused by *E. cloacae*, which was possibly caused by central venous catheter infection, and MEM was commenced on POD 106. The central venous catheter was removed on POD 109. *E. cloacae* was still isolated from blood culture despite 3 days of antibiotic therapy, and MEM was considered to be ineffective. The isolate was revealed to be a MBL producer and thus gentamycin was added on POD 116. However, the patient died on POD 117.

Case 3 was an 88-year-old man with an abdominal artery aneurysm for which an endovascular graft was inserted 6 months before admission. He was admitted to the hospital for colon cancer, and right hemicolectomy was performed. On POD 3, MEM was initiated for postoperative fever because of surgical site infection. Although his fever improved on POD 5, blood culture revealed MBL-producing *E. cloacae* and *Bacteroides* sp. Thus, MEM was switched to LVFX + ABPC/SBT on POD 7. Bacterial clearance was documented by a follow-up blood culture. The patient completed a 2-week course of intravenous antibiotic therapy followed by 2 weeks of oral LVFX + metronidazole and was discharged.

Drug susceptibility profiles are shown in Table 1. MIC values for both IPM and MEM were 2–4 μg/ml in all isolates, which were reported as susceptible in our clinical laboratory. All the isolates were positive for MBL by phenotypic, immunochromatographic, and polymerase chain reaction (PCR) assays. Two isolates produced IMP-1 and the remaining isolate produced IMP-11. There was no epidemiological link among the three patients.

Discussion

The reduced breakpoints of carbapenems for *Enterobacteriaceae* as revised recently by CLSI should be applied in clinical laboratories [4]. The MICs of IPM for all *Enterobacter cloacae* isolates from these cases were within the susceptible range according to the criteria recommended by CLSI in 2009 [8]. Nevertheless, these isolates were MBL producers. The breakpoint should have been ≤1 μg/ml for IPM in the present cases. CLSI recently recommended lowering the breakpoints for *Enterobacteriaceae* to improve the detection of carbapenemase producers [4]. However, higher MIC breakpoints are still used in many clinical laboratories, including those in Japan, because FDA-approved breakpoints have not been changed [5]. These higher breakpoints can lead to underestimation of the resistance, which may result in inadequate treatment. Yan et al. [9] recently reported that MBL production was not correlated with clinical outcomes and thus it was unnecessary to test MBL routinely. However, they did not analyze the association between MBL production and mortality by multivariate analysis. Information is still

Table 1 Susceptibility profiles of MBL-producing *Enterobacter cloacae* isolates

Isolates	MBL typing	MIC (μg/ml)									
		IPM	MEM	CTX	CAZ	CPR	AZT	P/T	CIP	AMK	CLS
1	IMP-1	2	2	512	512	6	64	64	1	1	2
2	IMP-11	2	2	32	64	4	0.5	64	32	8	2
3	IMP-1	2	4	256	512	16	32	64	0.5	1	2

IMP subtyping was performed by polymerase chain reaction and sequencing

MBL metallo-β-lactamase, *IPM* imipenem, *MEM* meropenem, *CTX* ceftriaxone, *CAZ* ceftazidime, *CPR* cefpirome, *AZT* aztreonam, *P/T* piperacillin/tazobactam, *CIP* ciprofloxacin, *AMK* amikacin, *CLS* colistin

scarce on this point, and further studies are needed to clarify whether MBL production is truly associated with poor outcome and should be tested routinely in clinical settings.

Tests for detecting MBLs may contribute to improved treatment of infections with carbapenem-resistant *Enterobacteriaceae*. These rapid tests include SMA Eiken (SMA, disk diffusion; Eiken Chemical) [10], Cica-β-test [6], PCR [11], and immunochromatographic assays [7]. Infections with MBL producers that have lower breakpoints than those presented by CLSI have been reported [12]. Therefore, additional methods may be required to accurately diagnose infections caused by MBL producers.

To our knowledge, this is the first report of IMP-type MBL-producing *E. cloacae* bloodstream infection in Japan, although a number of VIM-type MBL-producing *E. cloacae* infections have been reported in European countries [2, 3]. The Center for Disease Control and Prevention recommends active surveillance following isolation of carbapenemase-producing *Klebsiella* spp. or *Escherichia coli* because these isolates represent the majority of carbapenemase-producing *Enterobacteriaceae* in the United States [13]. However, active surveillance of *Enterobacter cloacae* is not included in this recommendation. More information is required to determine the validity of active surveillance of MBL-producing *E. cloacae* in healthcare facilities in Japan.

It is unclear whether carbapenems are effective against infections caused by IMP type MBL-producing *E. cloacae* showing MIC within the susceptible range. Two of our three cases were refractory to MEM, suggesting clinical inefficacy of carbapenems against MBL-producing *E. cloacae* regardless of their MIC. Falcone et al. [3] described seven cases of VIM-1-type MBL-producing *E. cloacae* infections: these cases were difficult to diagnose because of apparent susceptibility to carbapenems and were associated with high relapse rate and a prolonged duration of antibiotic therapy. Clinical studies on appropriate chemotherapies against MBL-producing *E. cloacae* infections will be required.

We reported three cases of MBL-producing *E. cloacae* showing relatively low MICs around the breakpoints for carbapenems. Effective testing strategies should be urgently implemented in medical facilities to adequately detect carbapenem-resistant *E. cloacae.*

Acknowledgments The authors thank all the clinical staff at Division of Infectious Diseases, Disease Control and Prevention Center for their clinical data and Kayo Shimada for performing susceptibility testing. This study was supported by a grant (H24-Shinko-ippan-003) from the Ministry of Health, Labor and Welfare of Japan.

Conflict of interest None declared.

Ethical approval Approved by Human Research Ethics Committee of National Center for Global Health and Medicine (NCGM/G/001232/00).

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J Infect Chemother 20 (2014) 586–588

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Note

Dissemination in Japan of multidrug-resistant *Pseudomonas aeruginosa* isolates producing IMP-type metallo-β-lactamases and AAC(6′)-Iae/AAC(6′)-Ib

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ARTICLE INFO

Article history:
Received 6 January 2014
Received in revised form
20 March 2014
Accepted 25 April 2014
Available online 6 June 2014

Keywords:
Pseudomonas aeruginosa
IMP
AAC
Multidrug-resistance
Immunochromatographic assay

ABSTRACT

The spread throughout Japan of antibiotic-resistance factors in multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolates was investigated epidemiologically, using immunochromatographic assays specific for IMP-type metallo-β-lactamases (IMPs) and aminoglycoside 6′-N-acetyltransferase [AAC(6′)]-Iae and -Ib. Three hundred MDR *P. aeruginosa* isolates were obtained during each of two years, 2011 and 2012, from 190 hospitals in 39 prefectures in Japan. The percentage of *P. aeruginosa* isolates producing IMPs, AAC(6′)-Iae or AAC(6′)-Ib increased significantly from 170/300 (56.7%) in 2011 to 230/300 (76.7%) in 2012, with 134/170 (78.8%) in 2011 and 179/230 (77.8%) in 2012 producing both IMP and either AAC(6′)-Iae or AAC(6′)-Ib. The MICs of antibiotics, including cephalosporins and carbapenems, were markedly higher for isolates that did than did not produce these resistance factors. These results indicated that MDR *P. aeruginosa* producing IMPs, AAC(6′)-Iae or AAC(6′)-Ib have spread throughout Japan and that these antibiotic-resistance factors are useful markers for monitoring MDR *P. aeruginosa* in Japan.

Although *Pseudomonas aeruginosa* is 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 [1]. Nosocomial outbreaks of *P. aeruginosa* infection, particularly by multidrug-resistant (MDR) strains, have become more frequent in various countries [2–5], including Japan [1,6].

MDR *P. aeruginosa* isolates in Japan frequently produce IMP-type metallo-β-lactamases (MBLs) and/or aminoglycoside 6′-N-acetyltransferases [AAC(6′)s]-Iae and -Ib [1,7–9]. We recently designed immunochromatographic assay kits for the detection of IMP-type MBLs and AAC(6′)-Iae and -Ib [10–12]. Clinical assessment showed that the results of these immunochromatographic assays were fully consistent with those of PCR analyses [10–12]. The aim of the study is to elucidate the spread of antibiotic-resistance factors in MDR *P. aeruginosa* isolates throughout Japan.

Bacterial species were identified with the MicroScan WalkAway system and MicroScan breakpoint panels (Siemens Healthcare Diagnostics, Tokyo, Japan). Drug susceptibility was determined qualitatively as sensitive (S), intermediate (I) or resistant (R) using MicroScan breakpoint panels (Siemens Healthcare Diagnostics) consistent with the guidelines of the Clinical and Laboratory Standards Institute (CLSI). MDR *P. aeruginosa* isolates were defined as isolates resistant to imipenem (IPM), amikacin (AMK) and ciprofloxacin (CPFX) using the breakpoint panels in the study. Minimum inhibitory concentrations (MICs) of CAZ, cefepime (CFPM), meropenem (MEMP), panipenem (PAPM), doripenem (DRPM), IPM, CPFX, levofloxacin (LVFX), AMK, and arbekacin (ABK) were determined by a broth microdilution method with dry plate (Eiken Chemical Co., Ltd., Tokyo, Japan). Values of MICs at which 50% and 90% of the isolates were inhibited (MIC_{50} and MIC_{90}, respectively) were determined.

Three hundred MDR *P. aeruginosa* isolates were obtained during each of two years (2011 and 2012) from single patients in 190

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http://dx.doi.org/10.1016/j.jiac.2014.04.014
1341-321X/

Table 1
Regions and sample origins where MDR *P. aeruginosa* strains were obtained in 2011 and 2012.

A

Year	Hokkaido/ Tohoku	Kanto/ Koshinetsu	Tokai/ Hokuriku/Kinki	Chugoku/ Shikoku	Kyushu/ Okinawa	Total
2011	53 (17.7%)	136 (45.3%)	71 (23.7%)	17 (5.7%)	23 (7.7%)	300
2012	40 (13.3%)	162 (54%)	67 (22.3%)	19 (6.3%)	19 (6.3%)	300

B

Year	Respiratory tract	Urinary tract	Others	Total
2011	134 (44.7%)	148 (49.3%)	18 (6%)	300
2012	124 (41.3%)	164 (54.7%)	12 (4%)	300

Distributions about regions (A) or sample origins (B) were not significantly different between 2011 and 2012.

hospitals located in 39 of the 47 prefectures in Japan. IMP-type MBLs, AAC(6′)-Iae, and AAC(6′)-Ib produced by these isolates were detected with immunochromatographic assay kits (Mizuho Medy Co., Saga, Japan) [10–12], as described [10]. Chi square tests were performed to compare the differences between data in 2011 and 2012.

Regions where samples were obtained were shown in Table 1A, according to the five regional blocks: i.e., Hokkaido/Tohoku, Kanto/Koshinetsu, Tokai/Hokuriku/Kinki, Chugoku/Shikoku, and Kyushu/Okinawa. Sample origins were also shown in Table 1B. Distributions about regions or sample origins were not significantly different between 2011 and 2012.

The distributions of MDR *P. aeruginosa* isolates producing IMP, AAC(6′)-Iae and AAC(6′)-Ib in 2011 and 2012 are shown in Table 2. Of the 300 isolates obtained during 2011, 170 (56.7%) were positive for the production of an IMP, AAC(6′)-Iae or AAC(6′)-Ib. In comparison, 230 of the 300 (76.7%) isolates obtained during 2012 were positive for the resistance factors, which was a significant increase over the rate in 2011 ($P < 0.01$). Of these positive isolates with at least more than one resistance factor, IMP and AAC(6′)-Iae co-producers were the most prevalent in 2011 and 2012. In addition, these IMP and AAC(6′)-Iae co-producers significantly increased from 28.7% of all MDR *P. aeruginosa* isolates tested in 2011 to 41.7% in 2012 ($P < 0.01$). Producers with other combinations of resistance factors did not increase or decrease significantly between 2011 and 2012.

Since most of the MDR *P. aeruginosa* isolates produced both IMP and AAC(6′)-Iae or both IMP and AAC(6′)-Ib, we compared the drug susceptibility of these isolates in 2012 with that of the isolates not producing these factors (Table 3). The MIC_{50} and MIC_{90} of cephalosporins and carbapenems were markedly higher for the isolates that did than did not produce these factors. There were no marked between group differences in the MIC_{50} and MIC_{90} of fluoroquinolones. The MIC_{50} of AMK, but not ABK, was significantly higher for isolates producing both IMP and AAC(6′)-Iae than for other groups. Similar results were observed for strains isolated in 2011 (data not shown).

Our study found that, of MDR *P. aeruginosa* isolates in Japan, IMP and AAC(6′)-Iae co-producers increased from 2011 to 2012 and showed higher MICs of cephalosporins and carbapenems than other groups. These producers also showed higher MIC of AMK, not ABK. These results were supported by a previous report describing that *Escherichia coli* DH5α expressing AAC(6′)-Iae was resistant to AMK but not to gentamicin and ABK [9].

We recently isolated MDR *P. aeruginosa* strains producing the novel aminoglycoside enzymes, AAC(6′)-Iaf [13] and AAC(6′)-Iaj [14]. Thin-layer chromatographic assay demonstrated that these enzymes effectively hydrolyzed AMK more effectively [13,14]. It is necessary to carefully monitor MDR *P. aeruginosa* isolates producing IMP-type metallo-β-lactamases, including novel IMP variants. These variants have been detected in MDR *P. aeruginosa* isolates, with one, IMP-43, conferring greater resistance to doripenem and meropenem but not to imipenem [7].

Use of these immunochromatographic assays has revealed various aspects of MDR *P. aeruginosa* prevalence and provided epidemiological information about drug resistance factors

Table 2
Drug resistance factors in MDR *P. aeruginosa* isolates in Japan.

Drug resistant factors							
Year	IMP+ AAC(6′)-Iae	IMP+ AAC(6′)-Ib	IMP	AAC(6′)-Iae	AAC(6′)-Ib	Negative	Total
2011	86 (28.7%)	48 (16%)	5 (1.6%)	2 (0.6%)	29 (9.7%)	130 (43.3%)	300
2012	125 (41.7%)	54 (18%)	11 (3.6%)	10 (3.3%)	30 (10%)	70 (23.3%)	300

Three hundred isolates were analyzed in each of two years, 2011 and 2012. Analysis showed that 130 isolates (43.3%) obtained in 2011 and 70 isolates (23.3%) in 2012 were negative for all the three factors, while 170 isolates (56.7%) and 230 isolates (76.7%), respectively, harbored at least one of the three factors.

Table 3
Drug susceptibility test of MDR *P. aeruginosa* isolates in 2012.

Drug resistant factors						
	IMP+ AAC(6′)-Iae		IMP+ AAC(6′)-Ib		Negative	
Antibiotics	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Cephalosporin						
CAZ	>128	>128	>128	>128	8	64
CEPM	>128	>128	>128	>128	16	64
Carbapenem						
MEPM	>128	>128	128	>128	16	64
PAPM	>128	>128	>128	>128	32	128
DPRM	>64	>64	>64	>64	8	32
IPM	128	>128	128	>128	16	32
Fluorquinolone						
CPFX	64	>128	32	128	32	64
LVFX	64	>128	64	128	64	128
Aminoglycoside						
AMK	128	128	32	64	32	64
ABK	8	32	16	64	16	64

associated with MDR *P. aeruginosa*. These immunochromatographic assays are simple methods that can rapidly detect antibiotic-resistance factors and are a useful alternative to PCR analysis for nationwide surveillance.

Author contributions

Experimental design: MT (Tojo), TT, TMA, TK, NO; Collection of clinical isolates and determination of MIC: MS; Immunochromatographic assay: MT (Tanaka), KN; Data analysis and preparation of the manuscript: MT (Tojo), TK, NO.

Conflicts of interest

Masahiro Shimojima is an employee of BML Inc., Masashi Tanaka and Kenji Narahara are employees of Mizuho Medy Co., Ltd. R&D. Their involvement does not alter our adherence to all the Journal policies on sharing data and materials. This study was not funded by those companies. The remaining authors have no reported potential conflicts of interest.

Acknowledgments

This study was supported by grants from International Health Cooperation Research (26-A-103) and a grant (H24-Shinko-ippan-010) from the Ministry of Health, Labor and Welfare of Japan.

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